A STABILITY AND SOLUBILITY STUDY OF RIBOFLAVIN AND SOME DERIVATIVES

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INTRODUCTION

Almost since the discovery and isolation of vitamin B_2 or riboflavin, the problem of solubility and stability in solution has been the cause of much concern.

Numerous methods have been suggested for preparing solutions containing a relatively high concentration of riboflavin. Most of these suggested methods, however, do not show any increase in stability greater than that of the pure vitamin itself. One of the purposes of this investigation was to prepare a more soluble form or derivative of this vitamin. Another aim was to prepare solutions of riboflavin, or a derivative thereof, which would be more stable to light and yet retain active physiological activity. An evaluation of riboflavin and some derivatives was made with regard to solubility and stability in various solvents and under different storage conditions.

It is often desirable to administer riboflavin parenterally, and to do so, it is necessary that the vitamin be present in a therapeutically effective amount and in a reasonable quantity of harmless diluent. It is likewise desirable to administer such riboflavin solutions by oral route. Such a form of vitamin B_2 could also be used for the enrichment of foodstuffs, for infant preparations and in many other pharmaceuticals.

Riboflavin is only very sparingly soluble in both water and in aqueous acidic solution. At 20° C. (1) only 0.12 mg. per ml. of water

will dissolve. Although more riboflavin is soluble in alkaline aqueous solutions, such solutions are extremely unstable and the riboflavin soon loses its physiological activity. It is of advantage to have a salt producing an acid pH present in the preparation of such solutions. This type of salt could serve to maintain the pH of the solution near the isoelectric point of riboflavin, thereby increasing its stability.

In human beings (2) natural as well as artificially induced riboflavin deficiencies have been observed. Lesions on the lips, and fissures at the angles of the mouth (cheilosis) are characteristic symptons which are promptly relieved by the administration of pure riboflavin. Human pellagra is often accompanied by definite symptoms of riboflavin deficiency, and in general, some riboflavin deficiency probably exists whether the outward symptoms are detectable or not.

Thus, it is apparent that the value of a concentrated and stable solution of vitamin B_2 is of prime importance. An attempt has been made in this investigation to prepare such types of solutions.

A REVIEW OF THE LITERATURE

Historical Sketch

The chemical nature of the yellow-green fluorescent pigment of whey, now referred to as riboflavin (synonymous with lactoflavin, vitamin G and vitamin B_2) commanded the attention of chemists (3) as early as 1879. A considerable concentration of this pigment was effected and certain of its more obvious chemical properties were clearly set forth by Bleyer and Kallmann (4) in 1925. No unusual significance was associated with this pigment by these early workers, who apparently regarded it only as one of the minor constituents of milk. The chemical nature of the pigment was still quite obscure.

In the course of an investigation into the nature of pellagra, Goldberger and Lillie (5) produced a deficiency disease in rats, characterized by ophthalmic and bilaterally symmetrical denuded areas. The factor that prevented these lesions was heat-stable, in contrast to vitamin B_1 which was heat-labile. It was termed by Goldberger, the P. P. (pellagra-preventing) factor but was later designated vitamin B_2 in Great Britain and vitamin G in the United States (6). It is now known that vitamin B_2 or riboflavin is not the rat pellagra-preventative factor but owing to the lack of knowledge at that time of the existence of other members of the B complex, this misconception was widely prevalent.

In 1932 Warburg and Christian (7) described a new oxidation enzyme obtained from aqueous extracts of yeast. The enzyme in water solutions was yellow and exhibited a green fluorescence. It has now been established (32) that this "yellow enzyme" is present in every living cell or at least in the cells of all the higher forms of life.

The symptoms reported by other workers as characteristic of vitamin B_2 deficiency varied considerably, however, and frequently differed markedly from those observed by Goldberger and Lillie (5). In particular, some workers reported only an absence of growth, while others noted the appearance of a dermatitis in some of the experimental animals.

In 1934 Gyorgy (8) (9) showed the fallacy of Goldberger and Lillie's experiments. This observer showed that rats maintained on a vitamin B free diet, with B_1 concentrate and lactoflavin added, developed a number of pellagra-like changes which were not only unrelieved, but even made worse by the addition of more vitamin B_2. These lesions, so produced, which were of a somewhat different character from those produced by Goldberger and Lillie, were cured by an unknown factor, tentatively named by Gyorgy vitamin B_6. It was contained in the "Peter's Eluate" from charcoal as prepared from yeast extract. This author admitted that certain skin lesions can be produced by deprivation of vitamin B_2 but made a sharp distinction between the manifestations so produced and those due to deprivation of vitamin B_6.

Thus, initially, the term vitamin B_2 was intended to describe the factor that caused pellagra, now known to be identical with nicotinic

acid. Subsequently, it came to be used to denote the rat growth factor, riboflavin.

The first step towards an understanding of the nature of vitamin B_2 was taken by Kuhn, Gyorgy and Wagner-Jauregg (10), who isolated from egg-white a compound with a strong yellowish-green fluorescence. They called this substance "ovoflavin" and showed that it stimulated the growth of rats.

In the same journal containing the paper by Kuhn et al., there appeared a paper by Ellinger and Koschara (11). They reported the presence of similar fluorescent substances in milk, liver, kidney, urine, muscle, yeast and in certain plant materials. They described the isolation of a crystalline fluorescent substance from whey. This substance obtained from whey they called "lactoflavin" and they proposed the name "lyochromes" for the group to which all these substances belonged. This term was in contradistinction to a group of naturally occurring fat-soluble pigments called "lypochromes." Both Kuhn et al. and Koschara (11) suggested that the pigments might be related to the "yellow enzyme" discovered in yeast. In fact, Kuhn showed that one and the same substance, lumiflavin, was produced by irradiation of the yellow enzyme and of riboflavin.

Shortly after the publication of these papers, Booher (12) reported the preparation of a concentrate from whey powder that showed a strong yellow fluorescence and had growth-promoting properties for the rat.

At first, these pigments isolated from various substances (13)

were given specific names according to their origin, for example: ovoflavin, lactoflavin, uroflavin and hepatoflavin. It was later realized that they were all probably identical with one another. This was confirmed by direct comparison of some of the compounds, but several were isolated in such small amounts that rigid proof of identity was not possible.

In 1936, at a conference group of the American Chemical Society meeting at Pittsburgh (14), the opinion was unanimous that the term flavin should be used to designate the water-soluble pigment that has been demonstrated to be necessary for the normal nutrition of the rat and for growing chicks. It was also determined at this meeting that the terms lactoflavin, vitamin G and vitamin B_2 should not be used. Riboflavin was to be the accepted name for vitamin B_2.

Isolation

Riboflavin has been isolated from a wide variety of animal and plant products (15) including egg-white, milk, liver, kidney, urine, barley malt, dandelion blossoms, grasses, egg yolk and retinas of fish eyes. It can be stated with absolute certainty that the crystalline flavin obtained from each of these various sources was chemically identical with riboflavin. At least such is the case for those to which adequate determinative tests have been applied.

The methods of isolation (16) varied somewhat in different laboratories and with the raw materials employed, but nearly all the workers used adsorption on fuller's earth (or in some instances lead sulfide) from a slightly acid-aqueous or aqueous-alcoholic extract. The resulting adsorbate was eluted with pyridine, or pyridine-methanol-water mixture or dilute ammonia, and the eluate, after being concentrated, was treated with a heavy metal, such as silver or thallium, to precipitate the flavin in the form of a salt. The free flavin was recovered from the precipitate by suitable treatment and recrystallized from water, dilute alcohol or dilute acetic acid.

In their earlier work, Kuhn and his co-workers (10) obtained from 100 Kg. of dried egg albumin, corresponding to about 33,000 eggs, 10 mg. of thrice recrystallized flavins. According to subsequent measurements (17) of the quantities of flavin normally present in dried egg albumin, this yield would correspond to about 7 per cent of the total flavin present in the egg albumin. Similarly the yield of crystalline

flavin from milk, as reported in the earlier work (18), was not greater than 5 per cent of the total quantity present. The use of heavy metal precipitation increased the yield to about 18 to 20 per cent of the quantity reported (17) to be normally present in milk.

Synthetic flavin was first prepared in 1934 by Karrer and Kuhn (19) and also by Reinemund and Weygand (20). This synthetic flavin was shown in both cases to be chemically identical with the flavin isolated from milk and to have the same biological value for rats. Karrer (21) first used the term riboflavin and its synthesis proved it to be an alloxazine structure combined with ribose.

Occurrence

Riboflavin (1) is widely distributed in nature in both plants and animals, being found as a free pigment or combined with a protein. It is an essential constituent of all living cells.

In the plant world, analysis (22) has shown that riboflavin occurs naturally in the green actively growing leaves and that it persists there in higher concentration than in other parts of the plant. Consequently, green stems and leaves are a much richer source of the vitamin than the flower or root. However, the vitamin is present in small amounts in practically all root vegetables and tubers.

There is reason to believe that as the leaves mature and dry, the riboflavin content may be correspondingly diminished (23). This may have a bearing on the vitamin content of milk since it has been found that cows fed on fresh young grass yield milk richer in riboflavin than animals receiving a more mature and drier grass (24). However, milk, either fresh or processed, seems to be a relatively rich and constant source of riboflavin.

The concentration of the vitamin in seeds (23) is subjected to considerable variation and reaches its maximum in the germ portion. Legumes, peas and beans provide a moderately rich source while nuts and cereal grains are somewhat poorer in their content. Fruits generally, and particularly citrus, have been proved to provide only a trifling amount of this substance.

The glandular organs of animals (24) constitute the richest of all foodstuffs in their riboflavin content. The lean muscle flesh

contains very considerable quantities.

Many species of microorganisms (25) are capable of synthesizing riboflavin, and because of the extensive bacterial growth in the human intestinal tract, this may form an important and constant source of supply.

The retinas of the eyes of many species of animals have been reported (26) to contain relatively high concentrations of flavin. It was supposed that the flavins are involved in some light sensitized reactions concerned with dim vision.

Riboflavin is synthesized commercially (1) on a large scale for addition to bread, flour and other dietary and pharmaceutical preparations.

Physical and Chemical Properties

Riboflavin (27) is a yellow to orange-yellow crystalline powder having a slight odor. It melts at about 280° C. and its saturated solution is neutral to litmus. Riboflavin is quite stable in strong mineral acids. When dry it is not appreciably affected by diffused light, but in solution, especially in the presence of alkali, it deteriorates quite rapidly, the deterioration being accelerated by light. Riboflavin is so sensitive to light that on irradiation with ultraviolet rays or visible light (1) it undergoes irreversible decomposition.

Riboflavin is 6,7-dimethyl-9-D-1'-ribitylisoalloxazine. It is thus a nitrogenous polyhydroxy alcohol (1).

At least one of the methyl groups in position 6 or 7 is essential in order that the flavin molecule shall possess vitamin activity. The absence of both the 6 and 7 methyl groups actually appears to be accompanied with toxicity (28). With regard to the side-chain, only the D-ribose or D-arabinose residue attached to the nitrogen atom in position 9 has thus far proved to be compatible with vitamin activity of the flavins. Exceedingly small variations in the side-chain often cause complete lack of vitamin activity.

The flavins, as a group, all share the tricyclic chromophoric nucleus that confers on them the yellow color to which they owe their group name. The vitamin activity (29) of the various members of this group of yellow pigments is profoundly influenced by the position and nature of the substituent groups in the benzene nucleus and by the nature of the side-chain attached to the pyrazine ring.

Riboflavin, the empirical formula of which is $C_{17}H_{20}N_4O_6$, has a solubility in water of 12 mg. per 100 ml. at 27.5° C. Some variations in solubility have been noted and this is due to differences in the internal crystalline structure of the vitamin. The aqueous solution has a strong yellowish-green fluorescence which is discharged by acid or alkali. This is used as a basis for identification by the U. S. P. (27).

Riboflavin (25) (27) is sparingly soluble in ethyl alcohol (4.5 mg. per 100 ml. at 27.5° C.) amyl alcohol, cyclohexanol, phenol or amyl acetate, but insoluble in acetone, ether, benzene or chloroform. It is more soluble in isotonic sodium chloride solution and very soluble in dilute alkali. By splitting off the D-ribityl side-chain (25) the resultant molecule becomes soluble in chloroform.

To increase the solubility of riboflavin in water (for injection use) the U. S. P. allows such preparations to contain nicotinamide, urea or other suitable harmless solubilizing agents.

In neutral or acid-aqueous solution, riboflavin (30) shows no rotation, but in alkaline solution it is strongly l-rotatory.

Riboflavin is amphoteric in nature, with an isoelectric point at pH 6 (31). The dissociation constants are:

$$K_a = 63 \times 10^{-12} \text{ and } K_b = 0.5 \times 10^{-5}.$$

On acetylation (10), a tetraacetate with a melting point of 242° C. is formed.

On irradiation in alkaline solution (32), riboflavin yields lumiflavin, $C_{13}H_{12}N_4O_2$, and this being sparingly soluble in water,

separates from the irradiated solution. Irradiation of neutral or acid solutions of riboflavin (33) is attended with the formation of 6,7-dimethyl-alloxazine or lumichrome which exhibits an intense blue fluorescence.

Goldblith and Proctor (34) showed that electrons or X-rays (3 megavolts from a Trump generator), which were used to irradiate solutions of pure riboflavin in metallic petri dishes, caused destruction of this fluorescent substance according to accepted methods of assay. The higher the concentration of irradiated vitamin, the less was the percentage of destruction. The products of irradiated riboflavin were lumichrome plus fragments.

Ellinger and Koschara (11) found vitamin B_2 to be reversibly reduced by sodium dithionite solution, by zinc in acid solution, by hydrogen sulfide in alkaline solution, by hydrogen in the presence of a catalyst, or by titanous chloride to a leuco-compound which was reoxidized to riboflavin and the color and fluorescence restored on exposure to air.

By maintaining riboflavin (35), both synthetic and natural, in a reduced state with sodium hydrosulfite, it can be protected from sunlight destruction. The reduced state can be reoxidized by vigorous shaking with excess air. Conclusions were that unreduced controls showed a 90 per cent destruction in a thirty minute exposure.

Riboflavin was said to be rendered more stable to light by the presence of sodium dithionite (36) or by heating with boric acid (37). Solutions containing boric acid are recommended for injection and are

said to be self-sterilizing as well as photo-stable.

Riboflavin (25) has a characteristic absorption spectrum, the peaks of the absorption bands being 221, 266, 359, and 445 mu.

Crystalline riboflavin is stable in the dark at ordinary temperatures but decomposes on exposure to light. Vitamin B_2 (38) is relatively heat-stable in acid solution, and the rate of destruction is rapidly increased with increasing alkalinity. In alkaline solutions it is unstable, especially when these solutions are exposed to light.

Ellinger and Holden (39) showed that at high concentrations of riboflavin in solution, the effect of "quenching" comes into play. It was considerably affected by certain anions, such as halides, cyanide, thiocyanide, sulfite and nitrite. Ferrous and ferric salts (an oxidation-reduction process) has a similar "quenching" effect.

Epley and Hall (40) experimented with several of the F. D. and C. colors and showed that riboflavin was unstable to F. D. and C. green number 3. However, F. D. and C. red number 3 and F. D. and C. orange number 1 seemed to protect vitamin B_2 from photochemical destruction.

Common cork, unless previously soaked in a large volume of water (41), contains a substance which strongly inhibits the fluorescence of riboflavin.

No appreciable destruction occurred when milk was incubated by Sure and Ford (42) for twenty-two hours at 31° to 37° C. or during the cooking of foods (43). When, on the other hand, milk in bottles was exposed to sunlight by Peterson et al. (44), more than half the riboflavin was destroyed within two hours.

Assay Determinations

The U. S. P. (27) gives both a microbiological assay procedure and a fluorophotometric method for determining riboflavin. The fluorophotometric method is based on the measure of fluorescence in acid solution. By comparing the concentration of an unknown solution with that of a prepared standard using a fluorophotometer that can accurately measure riboflavin activity in approximately 0.1 to 0.2 mcg. per ml., the amount present can accordingly be calculated. The microbiological method is carried out in acid media. It is also based on a comparison of an unknown with a standard solution using a pure culture of lactobacillus casei.

Visual methods for the determination of fluorescence have been employed, but photoelectric techniques (45) have been almost universally adopted in more recent years.

Loy (46) made a study of the fluorometric method and the microbiological method of assay of riboflavin. He found that there were no statistical differences between the results of the two methods.

When an amber transparent shade of the type commonly used in department store display windows for filtering out ultraviolet rays was placed over laboratory windows, it was found to minimize the destruction of riboflavin (47) by light rays during the course of assay. The use of added artificial light resulted in appreciable destruction of riboflavin.

So sensitive is riboflavin to the action of light that riboflavin assays should be carried out in dim light and preferably in red

light. De Merre and Brown (48) recommended a 150-watt lamp screened with a red cellophane filter. The light from the lamp normally employed in a Coleman spectrophotometer, however, does not cause appreciable destruction.

Various standards have been used for comparison with the intensity of fluorescence of the unknown, for example, pure riboflavin (49), potassium dichromate (50), fluorescein (51) and uranium glass (52) have been used as such standards.

Cohen (53) used a Kleinmann nephelometer with light from a mercury lamp filtered through a screen of nickel oxide for the determination of vitamin B_2 by means of its fluorescence.

To prepare a solution for fluorescent analysis, Weisberg and Levin (50) recommended the use of a clear solution. Various dilutions of this solution were made, up to 50 ml., in square 60 ml. bottles and compared under ultraviolet light with standards of sodium fluorescein. The standards were made up in terms of 0.1-1.0 mcg. of riboflavin per ml.

Riboflavin can be determined in concentrations of 0.005-2.0 mg. per liter as shown by Kavanagh (54). He used a two photocell balanced circuit with a galvanometer as a null point indicator to measure the ratio of the fluorescence of the unknown to that of a standard glass or quinine solution. With the use of proper filters, small amounts of suspended material did not interfere with the experiment.

For determining riboflavin content, Hodson and Norris (45) based their methods on the utilization of certain properties of ribo-

flavin. Basing their work on fluorometric methods their claims were:

1. Riboflavin fluoresced green when radiated with a blue light.
2. It was not destroyed by mild oxidation or reduction.
3. It could be reduced to a non-fluorescing form with sodium hydrosulfite and reoxidized readily by shaking with air.
4. It was not reduced by stannous chloride.
5. The intensity of the fluorescence could be measured with a photoelectric cell.

In further experimental work, Kuhn and Moruzzi (31), took measurements of the fluorescence of riboflavin solutions with graded pH values and showed that at a pH of 1.7 on the acid side and pH 10.2 on the alkaline side, the fluorescence brightness seemed to be proportional to the riboflavin concentration.

Jones and Christiansen (55) reported that riboflavin gave a maximum fluorescence at a pH of 6 to 7 whereas Karrer and Fritsche (56) pointed out that a maximum fluorescence was exhibited by a 0.003 per cent solution of riboflavin at pH 7.0.

Conner and Straub (57) showed that a linear relationship between fluorescence and concentration of riboflavin exists between the limits of 0.013 to 0.13 mcg. per ml.

Hanson and Weiss (58) recommended that in determining riboflavin concentrations, a standard solution containing 50 mcg. per ml. (50 parts per million) should be used. Although they found that it was difficult to get that much riboflavin into perfect solution, they

observed that fluorescence was proportional up to about 30 parts per million. In some cases the straight-line relationship between concentration and fluorescence would possibly continue up to 50 parts per million. It was said to be safer to work at a concentration not greater than 30 parts per million.

At the University of Witwatersrand in Johannesburg, Alper (59) claimed that substances which fluoresce exhibit fatigue when exposed continuously to the radiation which causes fluorescence. A dilute aqueous solution of riboflavin gave a straight line when the logarithm of the intensity of the fluorescent light was plotted against time. Every trial ended, not with an equilibrium state, but with a rate of fading which could no longer be measured. This photofatigue was important in the fluorometric assay of substances like riboflavin.

Slater and Morell (60), who worked with the Klett photoelectric fluorometer-colorimeter and the Klett photoelectric fluorometer, gave detailed procedures for making thiochrome and riboflavin solutions. It was shown that many precise measurements of the fluorescent intensity of solutions were possible with a fluorometer.

Increasing Solubility

In view of the low solubility of riboflavin, numerous methods have been suggested and many derivatives made for the preparation of solutions containing a relatively high concentration of the vitamin.

One of the earliest patents obtained to increase the solubility of riboflavin was received by Auhagen (61) in 1941. He claimed to have gotten up to 0.25 Gm. of riboflavin in 100 ml. of a 10 per cent nicotinamide or salts of nicotinic acid in water.

Frost (62) showed that riboflavin-nicotinamide solutions may be physiologically stabilized by adjusting the pH value of the solutions to 2.6-6.6 and preferably from 4.4-6.6. In a later work, Frost (63) proved that the solubility of riboflavin in nicotinamide solutions decreases progressively at pH values more acid than 5.0. As the nicotinamide concentration was increased from 5 to 50 per cent, the solubility of riboflavin increased at pH 5 from about 0.1 to about 2.5 per cent. The observed strong solvent effect of niacin on riboflavin appeared to be related to its chemical constitution with both C_5H_4N and $CONH_2$ groups being involved. An acid which formed an addition salt reduced the solvent action of nicotinamide but did not eliminate it.

Various details were given for the production of double salts of riboflavin, such as sodium riboflavin and an alkali metal borate by Auerbach (64). He claimed that the use of borax with an alkali was said to give the complex:

$$C_{17}H_{19}O_6N_4Na\text{-}Na_2B_4O_7.10H_2O.$$

In 1942, Frost (37) showed that a riboflavin-boron solution of good stability, containing up to 0.3 per cent riboflavin, can be obtained by heating an aqueous solution of riboflavin and up to 5 per cent boric acid at pH 6.5 for three hours at 95° C. With metaboric acid less heating was required. The specific rotation of riboflavin below pH 6 was enhanced in a positive direction by boron but the solvent effects of boron compounds were small below pH 6.0 and were increased above pH 6.0. It was found that the ribityl group was involved in the solvent reaction with boric acid and that the effect was independent of the very insoluble isoalloxazine group. Any attempt to benzoylate riboflavin in the presence of borates gave no reaction. Riboflavin tetrabenzoate and riboflavin monoborate were prepared. Isotonic preparations of the riboflavin-boron complex were self-sterilizing toward molds and bacteria and were suitable for injection.

Moran and Stein (65) experimented with the sodium salt of riboflavin and a polybasic carboxylic acid such as phthalic or succinic acid or their anhydrides refluxed in pyridine. After the reaction was completed the pyridine was evaporated, the residue dissolved in water and acidified. Crystals that separated were recrystallized from boiling water. These derivatives, particularly the succinate, were shown to have increased the solubility in water very favorably as compared to that of pure riboflavin.

Hoffer (66) showed that in 180 ml. of a 2 per cent solution of a lower alkylolamide of gentisic acid in water, he was able to get up to 0.33 Gm. of riboflavin soluble.

Hoffer in collaboration with Furter (67) showed that in aqueous solution containing 5 per cent gentisic acid and 5 per cent sodium gentisate at pH 5.0, riboflavin, up to 16 Gm. was soluble in each 100 ml.

Jurist (68) showed that concentrated riboflavin solutions were obtained by dissolving the vitamin in an aqueous solution of a pharmacodynamically unobjectionable aliphatic amidine acid addition product, such as acetamidine hydrochloride. A 20 per cent aqueous solution of acetamidine hydrochloride will carry 1900-2000 mcg. per ml. Solutions did not deposit riboflavin when chilled to 8° C. and they were heat-sterilized and stored without changes in color or clarity.

By using liver extract as a solvent, having 250 to 350 mg. per ml. of liver solids, Shelton (69) showed that it was possible to get up to 0.2 Gm. of riboflavin soluble per 100 ml.

Bird and Kuna (70) demonstrated that riboflavin was readily brought into solution with gallic acid or its alkali salts. Ten milliliters of a 10 per cent solution of gallic acid in 50 per cent aqueous ethyl alcohol dissolved 14 mg. of riboflavin. Sodium gallate in a 10 per cent aqueous solution at pH 6.7 dissolved 58 mg. of riboflavin in 10 ml. at 24.5° C. Dry mixtures of the vitamin and the salts of gallic acid dissolved readily in water.

Riboflavin was converted into a soluble complex by treatment with gallic acid in the presence of water and an inorganic acid by Zentner (71).

Preiswerk (72) reported a solubility of 4 Gm. of riboflavin per 100 ml. of an aqueous solution containing 25 per cent or more of a

water soluble salt of 2,4-dihydroxybenzoic acid or its lower monoalkyl ethers. The ortho, meta and para compounds of the latter were specified.

Water-soluble salts of benzoic acid and its amino or hydroxy-substituted derivatives were used as solubilizing agents in aqueous solutions by Miller (73). He showed that alkali benzoates (including sodium p-hydroxybenzoate and sodium p-aminobenzoate), magnesium or sodium salicylate and monoethanolamine salicylate all helped to increase the solubility of riboflavin.

Haas (74) claimed to have gotten up to 8.0 Gm. per ml. soluble as the citrate of diethylaminoacetyl riboflavin.

Upham (75) prepared stable, sterile and clear solutions of riboflavin citrate in propylene glycol. He reported up to 40 mg. per ml. as the possible solubility. Solutions containing additional substances in the same solvent were also given.

Moos and Upham (76) prepared citric, malic and tartaric acid esters of riboflavin by heating the acid and vitamin in phenol at 120° to 140° C. The esters were separated by pouring the cooled mixture into ether. The esters were water soluble and stable at pH 5.5-7.5 which were suitable for solutions for parenteral administration.

Knauf and Kirchmeyer (77) prepared solutions of riboflavin containing 0.1 to 0.3 per cent using water and 1 to 4 per cent veratyl alcohol as the solubilizer.

Solutions suitable for oral or parenteral administration and containing 0.15 to 0.3 per cent of riboflavin were reported by Charney (78). These solutions of riboflavin, alone or with other substances,

were prepared by using 1 per cent vanillin as the solubilizing agent in water or propylene glycol.

Charney (79) also reported the solubility of riboflavin in 4 per cent aqueous L-tyrosine amide at pH 5.0 and in 4 per cent aqueous L-tyrosine amide plus 10 per cent nicotinamide at pH 5.0.

The effect of sodium chloride, glycerin, urea, boric acid and sodium salicylate as solubilizers and stabilizers for concentrated solutions of riboflavin were studied by Gupta and Gupta (80). Boric acid was found to be the most efficient stabilizer but was painful when administered intramuscularly. Sodium salicylate (5 per cent) in a concentration of 2.5 mg. of riboflavin per ml. was found effective.

Gerlough and Smith (81) found that acetyltryptophan had a solubilizing action on riboflavin and could be used in the preparation of solutions for parenteral administration.

The solubility of riboflavin in aqueous media was increased by the addition of 20 parts of pyridylcarbinol (82) to 80 parts of water.

Schoen and Gordon (83) worked with water soluble methylol derivatives of riboflavin. By reacting formaldehyde with vitamin B_2, the monomethylol and dimethylol derivatives were obtained. Such compounds were found to be stable to potassium permanganate at room temperature but not at 50° C.

When riboflavin was fused with an amide, such as urea, urethan, acetamide or niacin, a product was obtained which yielded water solutions containing up to 6 per cent riboflavin. Stecher (84) showed that an acid salt, such as $NaH_2PO_4.H_2O$ may be incorporated in the melt or

added to the dry material. The composition of the fused product was not determined but it was assumed to contain equimolecular amounts of riboflavin and amide.

Stone (85) made a water-soluble derivative of riboflavin by dissolving it first in concentrated sulfuric acid, neutralizing with calcium hydroxide and freeze-drying. This calcium salt of a sulfate ester of riboflavin in aqueous solution was found to be stable in air and 1000 times as soluble as U. S. P. Riboflavin. It was soluble in methyl alcohol, glycerol and propylene glycol and slightly soluble in ethyl alcohol. Analysis indicated an empirical formula:

$$C_{17}H_{18}N_4O_{15}S_3Ca.$$

A portion of the sulfur was present as the sulfate. Fluorometric assay gave a riboflavin content of 57.2 per cent.

Riboflavin-5'-phosphate ester monosodium salt (86) was prepared by phosphorylation of riboflavin with chlorophosphoric acid. The solubility in water was claimed to be more than 100 times that of riboflavin. The empirical formula was reported as:

$$C_{17}H_{20}N_4O_9PNa.2H_2O.$$

It was fully active biologically, microbiologically and enzymatically. However, the greater sensitivity of the phosphate ester to destruction by ultraviolet light necessitated careful protection of dilute solutions from exposure. The monodiethanolamine salt, with a water solubility of more than 200 times that of riboflavin, was also prepared. This salt

was slightly acid in aqueous solutions.

A method of solubilizing riboflavin with sodium 3-hydroxy-2-naphthoate was developed by Arnold and Auerback et al. (87). In the procedure, the riboflavin itself was not treated. An aqueous solution with the naphthoate salt was prepared with the concentration being about double that of the desired concentration of riboflavin. The vitamin was then added and after a little stirring it went into solution. An exact mechanism of the solubilization effect was not proposed but it was believed that some sort of complex was formed.

EXPERIMENTAL

Materials Used

The drugs and chemicals used in this investigation together with the source, grade and lot number are shown in Table 1. The manufacturers of these materials are indicated by letters as follows:

L	Eli Lilly and Co.
M	Merck and Co., Inc.
W-S	Winthrop-Stearns, Inc.
H-LR	Hoffmann-La Roche, Inc.
MA	Matheson Co., Inc.
G	General Chemical Division
SA	Sargent Chemical Co.
E	Eastern Chemical Co.
S	Swift and Co.
D	Dow Chemical Co.
F	Fisher Scientific Co.
K	Koppers Co., Inc.
ML	Mallinckrodt Chemical Works
SNY	Smith New York
C	Carbide and Carbon Chemicals
B	Baker Chemical Co.
A	Atlas Powder Co.

TABLE 1

DESCRIPTION OF DRUGS AND CHEMICALS USED

Material	Manufacturer	Grade	Lot Number
Riboflavin	L M	USP USP	Sample 42989
Flavaxin Soluble (Riboflavin Sodium-Sodium Tetraborate)	W-S		R-023-KN
Riboflavin-5'-Phosphate Sodium	H-LR		510123
Pyruvic Acid	MA	CP	5300
Sodium Hydroxide	G	Reagent	J089
Barium Hydroxide	SA	Reagent	24392
Potassium Acid Phthalate	E	Reagent	92353
Fluorescein Sodium	M	USP	50223
Ethyl Ether	M	USP	0638
Glycerin	S	USP	800
Propylene Glycol	D	USP	1855550
Ethyl Aminobenzoate	F	USP	13847
Quinine Bisulfate	M	USP	43587
Beta-Methyl Umbelliferone	K		C5P-2132
Nicotinic Acid (Niacin)	ML	USP	2697
Citraconic Anhydride	SNY		Investigational
Liquefied Phenol	ML	USP	0024
Levulinic Acid (Liquid)	MA	CP	2404
Ethyl Alcohol	C	USP	

TABLE 1--Continued

Material	Manufacturer	Grade	Lot Number
Sodium Chloride	M	Reagent	41439
Potassium Chloride	M	Reagent	42378
Sodium Acid Phosphate	ML	USP	7868
Potassium Acid Phosphate	B	USP	2251
Nicotinamide	M	USP	50180
Urea	B	USP	1490005
Polyoxyethylene Sorbitan Monooleate (Tween 80)	A	USP	299

Preparation of Riboflavin Derivatives

Pyruvic Acid, Levulinic Acid and Citraconic Anhydride Derivatives

To 5 Gm. of riboflavin, placed in a 250-ml. red glass flask, were added 5 ml. of pyruvic acid, levulinic acid or citraconic anhydride (whichever was indicated), and 50 ml. of liquefied phenol. The flask was set in an oil bath and refluxed from four to six hours at a temperature range from 100-110° C. The reaction mixture was allowed to cool to room temperature and then poured, with constant stirring, into 500 ml. of ether in which the riboflavin derivative precipitated. The colored, crystalline precipitate was separated from the ether mixture by filtration on a Buchner funnel, and the product was washed with several portions of ether and dried between sheets of filter paper. The dried derivative was further purified by placing it in a mortar and triturating with a 100 ml. portion of ether and filtering. This procedure was repeated three times after which the preparation was again dried between sheets of filter paper. It was dried in an oven at 60° C. for four hours and then placed in the dark in a desiccator over phosphorous pentoxide. A working yield of derivative was obtained by this method.

Another method of synthesis, which did not prove as successful as that just mentioned, involved the refluxing of 2 Gm. of riboflavin and 8 ml. of the organic acid or anhydride in media made up of 2 ml. of concentrated sulfuric acid in 30 ml. of distilled water. This mixture was also refluxed in an oil bath at a temperature range from

100-110° C. for four to six hours. After the reaction mixture was cooled to room temperature, the sulfuric acid was carefully neutralized with a slurry of calcium oxide in water. The precipitated calcium sulfate was removed by filtration and the aqueous solution containing the derivative was evaporated to dryness in an oven at 60° C. This method was not generally employed in the preparation of soluble derivatives due to the apparently low riboflavin yields and the difficulty encountered with the isolation.

Other methods tried for isolating the riboflavin derivative from the reaction mixture were vacuum distillation and steam distillation. Although the compound was isolated with vacuum distillation, this procedure was found too time consuming. Steam distillation destroyed most of the vitamin.

Scope of the Fluorophotometer

The instrument used to determine the stability and solubility of riboflavin and some of its derivatives was the lumetron photoelectric fluorescence meter model 402-EF.

The operation of this type of fluorophotometer (88) is based on the light of a mercury vapor lamp which is condensed by an optical system to form a parallel beam. This beam is passed through a narrow-band filter which isolates the exciting light of the proper wave length. The exciting beam is split into two parts. One part enters the sample holder which is provided with a thin front window of low ultraviolet absorption. The fluorescence of the liquid is registered by two large barrier-layer photocells which are arranged laterally on both sides of the sample holder in the fluorescence pick-up unit. Filters between sample holder and photocells serve to isolate the specific fluorescence band and to eliminate the influence of primary light which may be scattered by particles suspended in the liquid. The other part of the beam is deflected by a front surface mirror and acts upon the balance which is mounted so that it can be turned through an angle of 90°. The two measuring photocells and the balance photocell are connected in a bridge circuit with a slide wire and with a galvanometer as the zero indicator. The purpose of the bridge circuit is to eliminate the influence of light intensity variations of the mercury vapor lamp.

The galvanometer was set to the zero mark in the center of the scale by means of the galvanometer zero adjustment knob. This setting was checked from time to time and readjusted when necessary. However,

it was not found necessary to have the light spot always exactly on the zero mark of the scale. The high sensitivity of the multiple reflection galvanometer made it necessary to treat the balancing operation in a manner differing slightly from the balancing of circuits employed in less sensitive galvanometers. A very convenient electrical method to accomplish this is used in the instrument. The middle position of the galvanometer key switch (off position) is made not to disconnect the galvanometer, but to connect the galvanometer and the other parts of the circuit into a substitute circuit in which all interferences but no photocurrents register on the galvanometer.

The lumetron is furnished with 10 bakelite plates numbered from 1 through 10. Plate 1 has no aperture whereas plates 2 through 10 are provided with apertures ranging in diameter from 1/16 of an inch to 3/4 of an inch. These plates fit into a slot on the right of the filter holder compartment and serve the purpose of reducing or blocking out the light on the balance photocell or on the measuring photocell. The most suitable reduction plate is selected by trying out the various apertures starting with the larger apertures and proceeding to the smaller ones. Finally, a very small aperture is found which no longer permits balancing even though the balance control is turned all the way counterclockwise. The reason for this is that with this reduction plate the balance beam has been reduced so much that the balance cell, even if turned to face the balance beam squarely, can no longer balance the current of the measuring cells. The smallest aperture with which it is still possible to balance the circuit or the next larger aperture should

be used. The selection of the aperture has no effect upon the fluorescence reading obtained but only upon the convenience in balancing with the balance cell controls.

In order to measure, by fluorescence, the concentration of an ingredient in a solution, it is necessary to have samples of the solvent alone as well as of the ingredient alone. A known amount of the ingredient is dissolved in the solvent and serves as the standard for which the instrument is balanced with the slide wire on 100. The solvent alone serves as the standard blank. If this blank shows a reading on the instrument (either due to inherent fluorescence of the solvent or due to scattered primary light), this reading is suppressed by means of a zero suppressor knob so as to make the blank read zero on the slide wire dial. The length of the slide wire scale from 0 to 100, then, covers the range of concentration from zero to the known concentration of the standard.

Standardization of the Lumetron

Preparation of Solutions

To prepare the lumetron for stability studies of riboflavin and some of its derivatives, it was necessary to adjust the instrument in such a manner so that there would be a linear relationship between concentration and fluorescence. Such a relationship exists in concentrations up to 2 mg. per liter (54). The plotting of a calibration curve is not necessary in this range, since the amount of fluorescence is directly proportional to the concentration.

The preparation of a standard stock solution of riboflavin was made by taking exactly 50 mg. of riboflavin, previously dried at 105° C. for two hours, and dissolving it in enough distilled water (acidified with 1 ml. of glacial acetic acid per liter) to make 1000 ml. To prepare a solution dilute enough for fluorophotometric analysis, this solution had to be further diluted by taking a 40 ml. aliquot and diluting with a sufficient amount of distilled water to make 1000 ml. The resulting concentration of this standard solution was 2 mg. per liter or 2 mcg. per ml.

Adjusting the Lumetron for Analysis

After setting the galvanometer to the zero mark, the primary and secondary filters were inserted into the lumetron. The sample holder, with 25 ml. of the riboflavin standard solution containing 2 mcg. per ml., was placed into the fluorescence pick-up unit. With the slide wire dial set at 100 and after the insertion of the proper reduction

plate, the lumetron balance cell was adjusted. This gave a reading of 100 with a solution containing 2 mcg. per ml.

The slide wire dial was then set at zero and a blank solution (solvent only) was placed in the pick-up unit. Here, the lumetron was adjusted to give a reading of zero by turning the suppressor knob counter-clockwise.

A solution containing 1 mcg. per ml. of fluorescein sodium was similarly prepared using distilled water. The fluorescence reading was taken after adjustment of the lumetron with the riboflavin solution and the blank. This fluorescence reading was used as a standard so that the instrument could be properly checked and adjusted each day before use.

After the lumetron was set for the maximum and minimum values, various dilutions were made of the standard riboflavin solution and fluorophotometric readings were taken as described in Table 2.

TABLE 2

LUMETRON READINGS OF VARIOUS DILUTIONS OF A STANDARD RIBOFLAVIN SOLUTION CONTAINING 2 MG./LITER

Ml. of Standard Riboflavin Solution	Ml. of Distilled Water Added	Mcg./Ml.	Lumetron Reading
0.50	24.50	0.04	1.5
1.25	23.75	0.1	4.7
2.50	22.50	0.2	9.8
3.75	21.25	0.3	15.0
5.00	20.00	0.4	20.5
6.25	18.75	0.5	25.3
7.50	17.50	0.6	30.5
8.75	16.25	0.7	35.5
10.00	15.00	0.8	40.5
11.25	13.75	0.9	45.3
12.50	12.50	1.0	50.1
13.75	11.25	1.1	55.3
15.00	10.00	1.2	60.5
16.25	8.75	1.3	65.3
17.50	7.50	1.4	70.2
18.75	6.25	1.5	75.2
20.00	5.00	1.6	80.4
21.25	3.75	1.7	84.8
22.50	2.50	1.8	90.0
23.75	1.25	1.9	95.0
25.00	0.00	2.0	100.0

Stability Study Methods

Solutions Used

The solutions used as solvents for the investigation of the stability of riboflavin and some of its derivatives were as follows:

Buffer Solution at pH 6.0

Buffer Solution at pH 5.0

Buffer Solution at pH 4.0

25 Per Cent Glycerin in Distilled Water

50 Per Cent Glycerin in Distilled Water

25 Per Cent Propylene Glycol in Distilled Water

50 Per Cent Propylene Glycol in Distilled Water

Saturated Solution of Ethyl Aminobenzoate in Distilled Water

0.01 Per Cent Quinine Bisulfate in Distilled Water

Saturated Solution of Beta-Methyl Umbelliferone in Distilled Water

1.0 Per Cent Urea in Distilled Water

0.1 Per Cent Tween 80 in Distilled Water

0.5 Per Cent Nicotinic Acid in Distilled Water

Buffer solutions from pH 4 to 6 were used in view of the literature reports that this range was most favorable to the stability of riboflavin solutions. Glycerin and propylene glycol in distilled water were selected because these are widely used vehicles in pharmacy. Aqueous solutions of ethyl aminobenzoate, quinine bisulfate and beta-methyl umbelliferone were used as solvents since it was thought that they might delay destruction of the vitamin in the presence of light due to their

sun screening properties. Both urea and nicotinic acid are used by some pharmaceutical houses as solubilizers for riboflavin, and it was thought these might be effective for stabilizing solutions of the riboflavin derivatives prepared in this investigation. Tween 80 was selected because it is commonly used in oral vitamin drops.

The distilled water used to make up all solutions throughout this investigation was laboratory distilled water. The pH varied from 6.1 to 6.4.

The solutions used in the preparation of buffer mixtures were prepared according to the Clark and Lubs procedure (27) as follows:

Barium Hydroxide Test Solution. A saturated solution of barium hydroxide was prepared by adding an excess amount to recently boiled distilled water, shaking thoroughly and then filtering. The test solution was freshly prepared each time it was needed.

0.2 M Sodium Hydroxide Solution. This solution was prepared by dissolving 9.00 Gm. of sodium hydroxide in 950 ml. of distilled water. A freshly prepared saturated solution of reagent barium hydroxide was added drop by drop until no more precipitate was formed. The mixture was thoroughly shaken and allowed to stand overnight in a stoppered bottle. The next day, the precipitate was filtered off and the resulting product gave a carbonate free solution of sodium hydroxide. To standardize the product, 10 ml. of normal sulfuric acid was diluted with 50 ml. of carbon dioxide free distilled water and two drops of phenolphthalein, T. S. was added. This solution was titrated with the sodium hydroxide solution until a permanent pink color was produced. The normality of

the sodium hydroxide solution was calculated and adjusted to exactly 0.2 M with freshly boiled and cooled distilled water.

0.2 M Potassium Biphthalate Solution. Exactly 40.843 Gm. of potassium biphthalate was dissolved in 900 ml. of distilled water and then sufficient distilled water added to make 1000 ml. The molarity was then determined and adjusted to exactly 0.2 M by titration with the prepared 0.2 M sodium hydroxide solution using phenolphthalein as the indicator.

0.2 M Monobasic Potassium Phosphate Solution. This preparation was made by dissolving exactly 27.218 Gm. of monobasic potassium phosphate in distilled water and diluting with sufficient distilled water to make 1000 ml. The molarity was determined and adjusted by titrating against 0.2 M sodium hydroxide solution.

The buffer solution at pH 6.0 was prepared by adding 50 ml. of 0.2 M monobasic potassium phosphate to 5.64 ml. of 0.2 M sodium hydroxide solution and diluting to 200 ml. with distilled water.

The buffer solution at pH 5.0 was prepared by taking 23.65 ml. of 0.2 M sodium hydroxide and adding it to 50 ml. of potassium biphthalate. This was diluted to 200 ml. with distilled water.

The buffer solution at pH 4.0 was prepared by adding 0.40 ml. of 0.2 M sodium hydroxide to 50 ml. of potassium biphthalate and diluting to 200 ml. with distilled water.

Solutions of glycerin, propylene glycol and polyoxyethylene sorbitan monooleate (Tween 80) in distilled water were prepared on a volume to volume basis whereas solutions of quinine bisulfate, urea and

nicotinic acid in distilled water were prepared on a weight to volume basis.

The saturated solution of ethyl aminobenzoate in distilled water was prepared by taking an amount of ethyl aminobenzoate that would normally dissolve in the desired quantity of water and dissolving it first in the smallest amount of ethyl alcohol. This alcoholic solution was then added to the distilled water a little at a time with thorough agitation. The preparation was allowed to stand overnight in a well stoppered bottle and then filtered the next day.

The saturated solution of beta-methyl umbelliferone in distilled water was prepared by adding 1 Gm. of beta-methyl umbelliferone to a liter of boiling distilled water with constant agitation, allowing it to cool to room temperature and setting it aside overnight in a well stoppered bottle. The next day the needle-like crystals which precipitated out of solution were removed by filtration.

Procedure

Stability studies were evaluated for the following preparations:

Riboflavin

Pyruvic Acid Derivative of Riboflavin

Levulinic Acid Derivative of Riboflavin

Flavaxin Soluble

Riboflavin-5'-Phosphate Sodium

Solutions of the above were prepared in different concentrations in 250-ml. red volumetric flasks. Each solution was kept in the dark

overnight in red colored and well stoppered bottles. An initial reading was taken just before the start of storage under various conditions of light.

Flint and amber bottles, commonly employed in the storage of pharmaceutical products, were used as the containers for the solutions. Three sets of each of the vitamin solutions were prepared for each solvent. One set of solutions was kept in direct sunlight by placing the containers on the flat roof of the building. Another set was kept in the diffused light of the laboratory by placing the containers on a table. The third set was kept in total darkness by setting the bottles in closed cardboard boxes in a laboratory desk locker.

Twenty-five milliliters were stored in each type of container. Approximately 1 ml. of toluene, enough to produce a layer on the top of the solution, was added to each container to prevent mold growth.

The lumetron was set to give a reading of 100 with a standard riboflavin solution containing 2 mcg. per ml. and a reading of zero with the particular solvent used. A certain quantity of the more concentrated solutions had to be diluted with enough distilled water to fall into the range of the lumetron.

Along with the storage of the solutions containing riboflavin or its derivatives, a set of blanks (the solvent only) was also stored under the same conditions. The blanks also were stored in amber and flint bottles with a layer of toluene on the top. The use of blanks was deemed necessary especially with solvents of 0.01 per cent quinine bisulfate in distilled water and with a saturated solution of beta-methyl

umbelliferone in distilled water which normally have their own inherent fluorescence. Accordingly, before a fluorophotometric reading was determined, the lumetron was adjusted to read zero with the insertion of the blank.

The vitamin content of all solutions in both flint and amber bottles was evaluated fluorophotometrically at the end of the following time intervals: one day, three days, five days, seven days, ten days, fifteen days, twenty days, thirty days and sixty days.

Riboflavin was used as a control in the stability study. Both Flavaxin Soluble and riboflavin-5'-phosphate sodium were selected because they are recognized as fairly soluble riboflavin derivatives and are available on the market. These were compared for stability with the riboflavin derivatives prepared in this investigation.

Solubility Study Methods

Solvents Used

The solvents and solutions used for the investigation of the solubility of riboflavin and some of its derivatives were as follows:

Distilled Water

Glycerin

Propylene Glycol

0.9 Per Cent Sodium Chloride in Distilled Water

0.9 Per Cent Potassium Chloride in Distilled Water

1.0 Per Cent Sodium Acid Phosphate in Distilled Water

1.0 Per Cent Potassium Acid Phosphate in Distilled Water

Ethyl Alcohol

1.0 Per Cent Niacinamide in Distilled Water

1.0 Per Cent Urea in Distilled Water

The above percentage solutions were prepared on a weight to volume basis. A sufficient quantity of salt was weighed out, dissolved in a portion of distilled water and then made up to volume with more distilled water.

Procedure

The method used for determining the solubility of riboflavin and some of its derivatives consisted of preparing saturated solutions in the solvents and solutions listed. Saturation was attained by placing an excess amount of riboflavin or a derivative thereof in the solvent,

heating to 60° C. for several minutes with constant agitation and then cooling to room temperature. Care was taken to avoid any unnecessary exposure to light in this procedure. After cooling, the preparations were well stoppered and stored overnight in total darkness. The next day, the excess amount of crystals was removed by centrifuging.

One milliliter of the solution was carefully pipetted into 950 ml. of distilled water and then further diluted to 1000 ml. with more distilled water. A 25-ml. aliquot of this solution was used to obtain a fluorophotometric reading. With the more soluble derivatives, it was found necessary to further dilute 1 ml. of the above solutions to 250 ml. in a red volumetric flask. This was sometimes found necessary because the lumetron was adjusted to give a reading of 100 with the standard riboflavin solution and a reading of zero with a distilled water blank. The preparation of the standard riboflavin solution was the same as that described under assay procedure.

Solubilities were determined for each of the following:

Riboflavin

Pyruvic Acid Derivative of Riboflavin

Levulinic Acid Derivative of Riboflavin

Citraconic Anhydride Derivative of Riboflavin

Flavaxin Soluble

Riboflavin-5'-Phosphate Sodium

Assay Method of Riboflavin Derivatives

The determination of the amount of riboflavin equivalent to a specific quantity of derivative was determined fluorophotometrically.

A standard riboflavin stock solution was first prepared. Riboflavin, U. S. P., was dried at 105° C. for two hours and stored in the dark in a desiccator over phosphorous pentoxide. Exactly 50 mg. were carefully weighed and dissolved in distilled water to make one liter. This solution was stored in a red glass bottle under toluene and placed in a refrigerator which was set at 5° C. Each ml. represented 50 mcg. of U. S. P. Riboflavin.

A standard riboflavin solution was prepared from the above stock solution by placing 10 ml. of the above preparation in a red glass volumetric flask and diluting to 250 ml. with distilled water. Each ml. represented 2 mcg. of riboflavin. A 25-ml. aliquot of this solution was placed in a sample container and the lumetron adjusted to read 100 with this concentration. The same amount of distilled water was used as the blank and the zero suppressor knob was adjusted to read zero.

Accordingly, the preparation of solutions of riboflavin derivatives were made in a similar manner as that described for riboflavin solutions. The resulting concentrations were also 2 mcg. per ml. After adjusting the lumetron with the standard riboflavin solution and the blank, a 25-ml. aliquot of the derivative solution was placed in a sample container. The percentage of riboflavin was determined directly by the amount of fluorescence registered on the lumetron.

The following derivatives of riboflavin were assayed in this manner:

Pyruvic Acid Derivative

Citraconic Anhydride Derivative

Levulinic Acid Derivative

Flavaxin Soluble

Riboflavin-5'-Phosphate Sodium

Results with Riboflavin

Stability studies were evaluated for riboflavin in various solvents and the results were used as a control for the derivatives synthesized in this investigation.

Fifty milligrams of riboflavin were added to a liter of each of the solvents listed under solubility studies. Since the lumetron was set for determinations up to 2 mcg. per ml., the riboflavin solutions were diluted by adding 1 ml. of the vitamin solutions to 24 ml. of distilled water. An initial lumetron reading was determined before the onset of storage.

Riboflavin was used as the basis for assay of the other derivatives studied in this investigation.

The solubility of riboflavin in various solvents was determined and the amount of riboflavin present in each ml. of saturated solution was evaluated fluorophotometrically.

TABLE 3

THE STABILITY OF RIBOFLAVIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 95.4 or 1.908 mcg./ml.						
One Day						
Amber	67.9	1.358	92.0	1.840	95.4	1.908
Flint	2.5	0.050	46.5	0.930		
Three Days						
Amber	43.0	0.860	90.5	1.810	95.4	1.908
Flint	2.0	0.040	40.0	0.800		
Five Days						
Amber	37.1	0.742	89.8	1.796	95.4	1.908
Flint	1.8	0.036	23.0	0.460		
Seven Days						
Amber	19.2	0.384	88.4	1.768	95.4	1.908
Flint	0.7	0.014	10.5	0.210		
Ten Days						
Amber	14.8	0.296	87.8	1.756	95.4	1.908
Flint	0.1	0.002	4.5	0.090		
Fifteen Days						
Amber	9.8	0.196	86.5	1.730	95.0	1.900
Flint	0.0	0.000	2.2	0.044		
Twenty Days						
Amber	3.5	0.090	76.2	1.524	95.0	1.900
Flint	...	.....	1.1	0.022		
Thirty Days						
Amber	2.0	0.040	71.5	1.430	94.5	1.890
Flint	...	.....	0.8	0.016		
Sixty Days						
Amber	0.0	0.000	54.3	1.086	93.4	1.868

TABLE 4

THE STABILITY OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 6 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 91.5 or 1.830 mcg./ml.						
One Day						
Amber	66.1	1.322	91.0	1.820	91.5	1.830
Flint	2.0	0.040	42.4	0.848		
Three Days						
Amber	53.2	1.064	90.1	1.802	91.5	1.830
Flint	1.4	0.028	38.9	0.778		
Five Days						
Amber	38.0	0.760	89.2	1.784	91.5	1.830
Flint	0.8	0.016	22.1	0.442		
Seven Days						
Amber	19.1	0.382	87.7	1.754	91.5	1.830
Flint	0.2	0.004	8.9	0.178		
Ten Days						
Amber	12.9	0.258	85.0	1.700	91.3	1.826
Flint	0.0	0.000	2.1	0.042		
Fifteen Days						
Amber	7.6	0.152	84.2	1.684	91.0	1.820
Flint	...	.....	0.6	0.012		
Twenty Days						
Amber	1.4	0.028	75.4	1.508	90.8	1.816
Flint	...	.....	0.3	0.006		
Thirty Days						
Amber	0.5	0.010	68.2	1.364	90.4	1.808
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	54.5	1.090	89.2	1.784

TABLE 5

THE STABILITY OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 5 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 91.2 or 1.824 mcg./ml.						
One Day						
Amber	66.4	1.328	91.0	1.820	91.2	1.824
Flint	2.2	0.044	40.2	0.804		
Three Days						
Amber	54.0	1.082	90.4	1.808	91.2	1.824
Flint	1.7	0.034	34.2	0.684		
Five Days						
Amber	40.1	0.802	90.0	1.800	91.2	1.824
Flint	0.9	0.018	23.2	0.464		
Seven Days						
Amber	19.1	0.382	88.7	1.774	91.2	1.824
Flint	0.0	0.000	9.0	0.180		
Ten Days						
Amber	14.8	0.296	86.1	1.722	91.2	1.824
Flint	...	.....	3.4	0.068		
Fifteen Days						
Amber	9.1	0.182	85.4	1.708	90.7	1.814
Flint	...	.....	1.1	0.022		
Twenty Days						
Amber	1.2	0.024	76.2	1.524	90.4	1.808
Flint	...	.....	0.4	0.008		
Thirty Days						
Amber	0.4	0.008	69.9	1.398	90.0	1.800
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	55.8	1.116	89.1	1.782

TABLE 6

THE STABILITY OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 4 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 93.3 or 1.866 mcg./ml.						
One Day						
Amber	68.2	1.364	93.3	1.866	93.3	1.866
Flint	2.0	0.040	43.1	0.862		
Three Days						
Amber	55.4	1.108	92.6	1.852	93.3	1.866
Flint	1.5	0.030	36.0	0.720		
Five Days						
Amber	40.1	0.802	91.8	1.836	93.3	1.866
Flint	1.0	0.020	24.2	0.484		
Seven Days						
Amber	20.0	0.400	90.9	1.818	93.3	1.866
Flint	0.2	0.004	9.2	0.182		
Ten Days						
Amber	15.2	0.304	90.1	1.802	93.3	1.866
Flint	0.0	0.000	2.2	0.044		
Fifteen Days						
Amber	10.9	0.218	89.4	1.788	92.9	1.858
Flint	...	.....	1.4	0.028		
Twenty Days						
Amber	2.5	0.050	79.8	1.596	92.5	1.850
Flint	...	.....	0.5	0.010		
Thirty Days						
Amber	1.1	0.022	75.0	1.500	92.0	1.840
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	60.1	1.202	91.3	1.826

TABLE 7

THE STABILITY OF RIBOFLAVIN IN 25 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 98.0 or 1.960 mcg./ml.						
One Day						
Amber	70.5	1.410	98.0	1.960	98.0	1.960
Flint	3.0	0.060	46.5	0.930		
Three Days						
Amber	50.4	1.008	94.5	1.890	98.0	1.960
Flint	1.0	0.020	40.1	0.802		
Five Days						
Amber	41.2	0.824	93.0	1.860	98.0	1.960
Flint	0.8	0.016	29.0	0.560		
Seven Days						
Amber	28.0	0.560	88.9	1.778	98.0	1.960
Flint	0.3	0.006	18.2	0.364		
Ten Days						
Amber	12.8	0.256	88.3	1.766	98.0	1.960
Flint	0.0	0.000	9.2	0.184		
Fifteen Days						
Amber	7.4	0.148	87.3	1.746	98.0	1.960
Flint	...	.....	5.0	0.100		
Twenty Days						
Amber	4.6	0.092	77.1	1.542	97.7	1.954
Flint	...	.....	3.0	0.060		
Thirty Days						
Amber	3.0	0.060	71.5	1.430	97.2	1.944
Flint	...	.....	1.4	0.028		
Sixty Days						
Amber	0.0	0.000	60.2	1.204	96.0	1.920

TABLE 8

THE STABILITY OF RIBOFLAVIN IN 50 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 92.0 or 1.840 mcg./ml.						
One Day						
Amber	80.5	1.610	91.0	1.820	92.0	1.840
Flint	2.5	0.050	53.5	1.070		
Three Days						
Amber	59.0	1.180	90.2	1.804	92.0	1.840
Flint	1.0	0.020	46.0	0.920		
Five Days						
Amber	49.6	0.992	89.1	1.782	92.0	1.840
Flint	0.7	0.140	35.2	0.704		
Seven Days						
Amber	38.4	0.768	88.3	1.766	92.0	1.840
Flint	0.1	0.002	24.2	0.484		
Ten Days						
Amber	34.6	0.692	86.1	1.722	92.0	1.840
Flint	0.0	0.000	13.2	0.264		
Fifteen Days						
Amber	29.8	0.596	84.5	1.690	91.8	1.836
Flint	...	.....	9.4	0.188		
Twenty Days						
Amber	21.5	0.430	76.1	1.522	91.6	1.832
Flint	...	.....	7.4	0.148		
Thirty Days						
Amber	15.2	0.304	72.3	1.446	91.3	1.826
Flint	...	.....	5.0	0.100		
Sixty Days						
Amber	0.0	0.000	59.8	1.196	91.0	1.820

TABLE 9

THE STABILITY OF RIBOFLAVIN IN 25 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 94.8 or 1.896 mcg./ml.						
One Day						
Amber	63.5	1.270	94.0	1.880	94.8	1.896
Flint	1.5	0.030	42.1	0.842		
Three Days						
Amber	42.8	0.856	92.0	1.840	94.8	1.896
Flint	0.8	0.016	33.5	0.670		
Five Days						
Amber	37.6	0.752	90.6	1.812	94.8	1.896
Flint	0.1	0.002	24.5	0.490		
Seven Days						
Amber	23.1	0.462	89.2	1.784	94.8	1.896
Flint	0.0	0.000	16.1	0.322		
Ten Days						
Amber	9.4	0.188	88.1	1.762	94.8	1.896
Flint	...	.....	12.0	0.240		
Fifteen Days						
Amber	5.8	0.116	88.0	1.760	94.4	1.888
Flint	...	.....	6.3	0.126		
Twenty Days						
Amber	4.1	0.082	80.2	1.604	94.0	1.840
Flint	...	.....	5.2	0.104		
Thirty Days						
Amber	2.0	0.040	77.4	1.548	93.7	1.874
Flint	...	.....	3.8	0.076		
Sixty Days						
Amber	0.0	0.000	56.8	1.136	93.0	1.860

TABLE 10

THE STABILITY OF RIBOFLAVIN IN 50 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 89.5 or 1.790 mcg./ml.						
One Day						
Amber	82.5	1.650	88.5	1.770	89.5	1.790
Flint	1.5	0.030	49.0	0.980		
Three Days						
Amber	62.3	1.246	86.1	1.722	89.5	1.790
Flint	1.1	0.022	41.2	0.824		
Five Days						
Amber	48.9	0.978	84.0	1.680	89.5	1.790
Flint	0.3	0.006	29.8	0.598		
Seven Days						
Amber	40.2	0.804	83.2	1.664	89.5	1.790
Flint	0.0	0.000	16.4	0.328		
Ten Days						
Amber	33.2	0.664	81.8	1.636	89.5	1.790
Flint	...	.....	10.1	0.202		
Fifteen Days						
Amber	25.8	0.516	80.1	1.602	89.1	1.782
Flint	...	.....	4.9	0.098		
Twenty Days						
Amber	19.0	0.380	78.8	1.576	89.0	1.780
Flint	...	.....	4.0	0.080		
Thirty Days						
Amber	12.1	0.242	76.8	1.536	88.7	1.774
Flint	...	.....	3.0	0.060		
Sixty Days						
Amber	0.0	0.000	54.3	1.086	88.0	1.760

TABLE 11

THE STABILITY OF RIBOFLAVIN IN A SATURATED SOLUTION OF ETHYL AMINOBENZOATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 90.0 or 1.800 mcg./ml.						
One Day						
Amber	75.5	1.510	90.0	1.800	90.0	1.800
Flint	4.0	0.080	67.0	1.340		
Three Days						
Amber	45.0	0.900	88.1	1.762	90.0	1.800
Flint	2.8	0.056	61.5	1.230		
Five Days						
Amber	34.1	0.682	87.5	1.750	90.0	1.800
Flint	0.7	0.014	54.8	1.096		
Seven Days						
Amber	28.2	0.564	87.2	1.744	90.0	1.800
Flint	0.2	0.004	40.6	0.812		
Ten Days						
Amber	23.7	0.474	87.0	1.740	90.0	1.800
Flint	0.0	0.000	40.1	0.802		
Fifteen Days						
Amber	12.1	0.242	84.6	1.692	90.0	1.800
Flint	...	.....	20.3	0.406		
Twenty Days						
Amber	6.4	0.128	80.5	1.610	90.0	1.800
Flint	...	.....	7.3	0.146		
Thirty Days						
Amber	0.5	0.010	77.2	1.544	89.5	1.790
Flint	...	.....	2.5	0.050		
Sixty Days						
Amber	0.0	0.000	69.2	1.384	88.5	1.770

TABLE 12

THE STABILITY OF RIBOFLAVIN IN 0.01 PER CENT QUININE BISULFATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 90.0 or 1.800 mcg./ml.						
One Day						
Amber	68.0	1.360	90.0	1.800	90.0	1.800
Flint	17.1	0.342	47.0	0.940		
Three Days						
Amber	65.0	1.300	85.4	1.708	90.0	1.800
Flint	10.1	0.202	44.2	0.884		
Five Days						
Amber	53.6	1.072	84.2	1.684	90.0	1.800
Flint	1.5	0.030	36.3	0.726		
Seven Days						
Amber	37.8	0.756	84.0	1.680	90.0	1.800
Flint	1.0	0.020	26.8	0.536		
Ten Days						
Amber	33.4	0.668	83.0	1.660	90.0	1.800
Flint	0.3	0.006	10.8	0.216		
Fifteen Days						
Amber	30.2	0.604	82.1	1.642	90.0	1.800
Flint	0.0	0.000	3.1	0.062		
Twenty Days						
Amber	21.0	0.420	81.4	1.628	89.4	1.788
Flint	...	.....	1.5	0.030		
Thirty Days						
Amber	10.6	0.212	76.2	1.524	89.2	1.784
Flint	...	.....	0.5	0.010		
Sixty Days						
Amber	0.0	0.000	63.4	1.268	88.0	1.760

TABLE 13

THE STABILITY OF RIBOFLAVIN IN A SATURATED SOLUTION OF BETA-METHYL UMBELLIFERONE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 90.5 or 1.810 mcg./ml.						
One Day						
Amber	69.0	1.380	90.5	1.810	90.5	1.810
Flint	2.2	0.044	67.5	1.350		
Three Days						
Amber	43.2	0.864	90.0	1.800	90.5	1.810
Flint	1.2	0.024	63.7	1.274		
Five Days						
Amber	32.1	0.642	87.1	1.742	90.5	1.810
Flint	0.8	0.016	54.0	1.080		
Seven Days						
Amber	28.5	0.570	85.8	1.716	90.5	1.810
Flint	0.1	0.002	43.2	0.864		
Ten Days						
Amber	14.1	0.282	84.5	1.690	90.5	1.810
Flint	0.0	0.000	22.9	0.458		
Fifteen Days						
Amber	6.1	0.122	82.0	1.640	90.5	1.810
Flint	...	.....	15.4	0.308		
Twenty Days						
Amber	4.2	0.084	75.0	1.500	90.0	1.800
Flint	...	.....	4.2	0.084		
Thirty Days						
Amber	0.2	0.040	71.0	1.420	89.8	1.796
Flint	...	.....	0.1	0.002		
Sixty Days						
Amber	0.0	0.000	63.1	1.262	88.6	1.772

TABLE 14

THE STABILITY OF RIBOFLAVIN IN 1.0 PER CENT UREA IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 92.0 or 1.840 mcg./ml.						
One Day						
Amber	64.5	1.290	92.0	1.840	92.0	1.840
Flint	1.5	0.030	40.1	0.802		
Three Days						
Amber	44.8	0.896	90.0	1.800	92.0	1.840
Flint	0.8	0.016	32.1	0.642		
Five Days						
Amber	33.0	0.660	85.2	1.704	92.0	1.840
Flint	0.0	0.000	14.8	0.296		
Seven Days						
Amber	17.0	0.340	79.4	1.588	92.0	1.840
Flint	0.0	0.000	6.5	0.130		
Ten Days						
Amber	4.5	0.090	75.2	1.504	91.5	1.830
Flint	...	.....	2.1	0.042		
Fifteen Days						
Amber	2.4	0.048	69.8	1.396	91.3	1.826
Flint	...	.....	1.2	0.024		
Twenty Days						
Amber	0.8	0.016	62.5	1.250	91.0	1.820
Flint	...	.....	0.8	0.016		
Thirty Days						
Amber	0.1	0.002	53.1	1.062	91.0	1.820
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	42.8	0.856	90.4	1.808

TABLE 15

THE STABILITY OF RIBOFLAVIN IN 0.1 PER CENT TWEEN 80 IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 91.0 or 1.820 mcg./ml.						
One Day						
Amber	62.8	1.256	88.0	1.760	91.0	1.820
Flint	4.0	0.080	40.5	0.810		
Three Days						
Amber	41.9	0.838	86.1	1.722	91.0	1.820
Flint	2.8	0.056	35.0	0.700		
Five Days						
Amber	32.0	0.640	85.2	1.704	91.0	1.820
Flint	1.4	0.028	20.1	0.402		
Seven Days						
Amber	15.8	0.316	83.8	1.676	91.0	1.820
Flint	0.8	0.016	8.5	0.170		
Ten Days						
Amber	2.7	0.054	82.5	1.650	91.0	1.820
Flint	0.4	0.008	4.1	0.082		
Fifteen Days						
Amber	1.8	0.036	80.2	1.604	90.7	1.814
Flint	0.0	0.000	0.8	0.016		
Twenty Days						
Amber	0.4	0.008	71.1	1.422	90.4	1.808
Flint	...	.....	0.3	0.006		
Thirty Days						
Amber	0.1	0.002	65.1	1.302	89.8	1.796
Flint	...	.....	0.0	0.000		
Sixty Days	0.0	0.000	51.7	1.034	88.9	1.778

TABLE 16

THE STABILITY OF RIBOFLAVIN IN 0.5 PER CENT NIACIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 94.2 or 1.884 mcg./ml.						
One Day						
Amber	68.8	1.376	94.0	1.880	94.2	1.884
Flint	1.0	0.020	40.0	0.800		
Three Days						
Amber	53.8	1.076	93.1	1.862	94.2	1.884
Flint	0.6	0.012	34.2	0.684		
Five Days						
Amber	39.2	0.784	92.4	1.848	94.2	1.884
Flint	0.2	0.004	22.0	0.440		
Seven Days						
Amber	17.9	0.358	91.1	1.822	94.2	1.884
Flint	0.0	0.000	7.2	0.144		
Ten Days						
Amber	13.1	0.262	89.4	1.788	94.2	1.884
Flint	...	.....	1.0	0.020		
Fifteen Days						
Amber	8.9	0.178	88.2	1.764	93.9	1.878
Flint	...	.....	0.4	0.008		
Twenty Days						
Amber	2.1	0.042	79.0	1.580	93.4	1.868
Flint	...	.....	0.0	0.000		
Thirty Days						
Amber	0.8	0.016	74.2	1.484	93.0	1.860
Flint	...	.....	...	.....		
Sixty Days						
Amber	0.0	0.000	59.2	1.184	92.1	1.842

TABLE 17

THE SOLUBILITY OF RIBOFLAVIN IN SOME AQUEOUS SOLUTIONS AND OTHER SOLVENTS

	Average Lumetron Reading	Mg./Ml.
Distilled Water	7.2	0.14
0.9% Sodium Chloride	10.0	0.20
0.9% Potassium Chloride	9.5	0.19
1.0% Sodium Acid Phosphate	5.5	0.11
1.0% Potassium Acid Phosphate	5.5	0.11
1.0% Niacinamide	20.0	0.40
1.0% Urea	17.0	0.34
Propylene Glycol	26.4	0.52
Glycerin	42.0	0.84
Alcohol	2.2	0.04

Results with Riboflavin-5'-Phosphate Sodium

Hoffmann-La Roche's riboflavin-5'-phosphate sodium salt was selected for study because of its unusually high solubility and its availability on the market.

The assay of this salt by the fluorophotometric procedure showed a 70.0 per cent riboflavin content. Thus, 1 Gm. of riboflavin-5'-phosphate sodium was equivalent to 0.7 Gm. of riboflavin.

Five milligrams of riboflavin-5'-phosphate sodium were added to each ml. of the solvents used for stability study. Since the lumetron was set for determinations up to 2 mcg. per ml., each solution had to be further diluted by adding 0.4 ml. to a sufficient quantity of distilled water to make 1000 ml. Twenty-five milliliters of this dilution were used for fluorophotometric analysis.

The solubility of Hoffman-La Roche's product in some aqueous solutions and other solvents was determined. Due to its unusually high solubility further dilutions with distilled water were necessary for evaluation with the lumetron.

TABLE 18

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 70.0 or 1.400 mcg./ml.						
One Day						
Amber	15.2	0.304	66.2	1.324	70.0	1.400
Flint	1.0	0.020	33.0	0.660		
Three Days						
Amber	3.5	0.070	64.1	1.282	70.0	1.400
Flint	0.1	0.002	16.1	0.322		
Five Days						
Amber	2.0	0.040	60.4	1.208	70.0	1.400
Flint	0.0	0.000	1.8	0.036		
Seven Days						
Amber	1.5	0.030	59.8	1.196	70.0	1.400
Flint	...	.....	1.0	0.020		
Ten Days						
Amber	1.0	0.020	57.8	1.156	70.0	1.400
Flint	...	.....	0.6	0.012		
Fifteen Days						
Amber	0.4	0.008	54.5	1.090	69.7	1.394
Flint	...	.....	0.1	0.002		
Twenty Days						
Amber	0.0	0.000	52.8	1.056	68.5	1.370
Flint	...	.....	0.0	0.000		
Thirty Days						
Amber	...	.....	49.4	0.988	67.7	1.354
Flint	...	.....	...	.....		
Sixty Days						
Amber	...	.....	38.6	0.772	65.5	1.310

TABLE 19

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN DISTILLED WATER BUFFERED AT pH 6 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 72.5 or 1.450 mcg./ml.						
One Day						
Amber	17.5	0.350	67.1	1.342	72.5	1.450
Flint	1.8	0.036	34.1	0.682		
Three Days						
Amber	4.8	0.096	63.8	1.276	72.5	1.450
Flint	0.6	0.012	17.1	0.342		
Five Days						
Amber	2.5	0.050	62.1	1.242	72.5	1.450
Flint	0.0	0.000	2.8	0.056		
Seven Days						
Amber	1.8	0.036	59.8	1.196	72.5	1.450
Flint	...	.....	1.5	0.030		
Ten Days						
Amber	1.1	0.022	57.4	1.148	72.0	1.440
Flint	...	.....	1.0	0.020		
Fifteen Days						
Amber	0.6	0.012	56.0	1.120	71.6	1.432
Flint	...	.....	0.4	0.008		
Twenty Days						
Amber	0.0	0.000	54.0	1.088	70.9	1.418
Flint	...	.....	0.0	0.000		
Thirty Days						
Amber	...	.....	51.6	1.032	70.0	1.400
Flint	...	.....	...	.....		
Sixty Days						
Amber	...	.....	40.3	0.806	67.8	1.356

TABLE 20

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN DISTILLED WATER BUFFERED AT pH 5 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 74.2 or 1.484 mcg./ml.						
One Day						
Amber	18.7	0.374	73.0	1.460	74.2	1.482
Flint	2.0	0.040	35.0	0.700		
Three Days						
Amber	5.0	0.100	71.4	1.428	74.2	1.482
Flint	0.8	0.016	18.5	0.370		
Five Days						
Amber	3.1	0.062	69.5	1.390	74.2	1.482
Flint	0.2	0.004	3.3	0.066		
Seven Days						
Amber	1.5	0.030	67.6	1.352	74.2	1.482
Flint	0.0	0.000	2.3	0.046		
Ten Days						
Amber	1.0	0.020	63.2	1.264	73.8	1.476
Flint	...	.....	1.8	0.036		
Fifteen Days						
Amber	0.7	0.014	58.5	1.170	73.3	1.466
Flint	...	.....	0.6	0.012		
Twenty Days						
Amber	0.0	0.000	55.5	1.116	72.6	1.452
Flint	...	.....	0.2	0.004		
Thirty Days						
Amber	...	.....	53.4	1.068	72.0	1.440
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	...	.....	42.5	0.850	70.1	1.402

TABLE 21

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN DISTILLED WATER BUFFERED AT pH 4 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 73.6 or 1.472 mcg./ml.						
One Day						
Amber	18.0	0.360	72.0	1.440	73.6	1.472
Flint	1.8	0.036	42.5	0.850		
Three Days						
Amber	4.7	0.094	70.4	1.408	73.6	1.472
Flint	1.0	0.020	17.1	0.342		
Five Days						
Amber	2.8	0.056	69.5	1.390	73.6	1.472
Flint	0.4	0.008	5.6	0.112		
Seven Days						
Amber	1.3	0.026	68.0	1.360	73.6	1.472
Flint	0.0	0.000	3.8	0.076		
Ten Days						
Amber	0.8	0.016	64.2	1.284	73.0	1.460
Flint	...	.....	1.5	0.030		
Fifteen Days						
Amber	0.2	0.004	62.2	1.244	72.4	1.448
Flint	...	.....	1.0	0.020		
Twenty Days						
Amber	0.0	0.000	60.5	1.210	71.9	1.438
Flint	...	.....	0.0	0.000		
Thirty Days						
Amber	...	.....	55.0	1.100	71.5	1.430
Flint	...	.....	...	.....		
Sixty Days						
Amber	...	.....	44.0	0.880	69.8	1.398

TABLE 22

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 25 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 73.9 or 1.478 mcg./ml.						
One Day						
Amber	23.1	0.462	73.0	1.460	73.9	1.478
Flint	2.1	0.042	57.5	1.150		
Three Days						
Amber	10.1	0.202	72.8	1.456	73.9	1.478
Flint	1.4	0.028	40.7	0.814		
Five Days						
Amber	2.1	0.042	72.1	1.442	73.9	1.478
Flint	0.7	0.014	26.6	0.532		
Seven Days						
Amber	1.8	0.036	71.0	1.420	73.9	1.478
Flint	0.1	0.002	18.8	0.376		
Ten Days						
Amber	1.5	0.030	67.0	1.340	73.3	1.476
Flint	0.0	0.000	8.0	0.160		
Fifteen Days						
Amber	0.9	0.018	63.5	1.270	72.8	1.456
Flint	...	.....	2.3	0.026		
Twenty Days						
Amber	0.3	0.006	60.8	1.216	72.6	1.452
Flint	...	.....	0.9	0.018		
Thirty Days						
Amber	0.0	0.000	54.1	1.082	72.4	1.448
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	...	.....	44.8	0.896	70.1	1.402

TABLE 23

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 50 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 69.3 or 1.386 mcg./ml.						
One Day						
Amber	23.8	0.476	69.0	1.380	69.3	1.386
Flint	2.2	0.044	46.1	0.922		
Three Days						
Amber	16.1	0.322	68.3	1.366	69.3	1.386
Flint	1.3	0.016	29.1	0.582		
Five Days						
Amber	8.9	0.178	67.8	1.356	69.3	1.386
Flint	0.6	0.012	20.6	0.412		
Seven Days						
Amber	4.6	0.092	66.0	1.320	69.3	1.386
Flint	0.2	0.004	18.2	0.364		
Ten Days						
Amber	3.0	0.060	62.1	1.242	69.3	1.386
Flint	0.0	0.000	12.5	0.250		
Fifteen Days						
Amber	2.1	0.042	61.1	1.222	68.9	1.378
Flint	...	.....	9.1	0.182		
Twenty Days						
Amber	1.1	0.022	59.6	1.192	68.5	1.370
Flint	...	.....	6.6	0.132		
Thirty Days						
Amber	0.0	0.000	56.3	1.126	67.8	1.356
Flint	...	.....	3.2	0.064		
Sixty Days						
Amber	...	.....	48.5	0.970	66.4	1.328

TABLE 24

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 25 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 72.0 or 1.440 mcg./ml.						
One Day						
Amber	19.0	0.380	67.7	1.354	72.0	1.440
Flint	1.9	0.038	52.3	1.046		
Three Days						
Amber	9.2	0.184	65.7	1.314	72.0	1.440
Flint	1.0	0.020	28.6	0.572		
Five Days						
Amber	2.0	0.040	64.2	1.284	72.0	1.440
Flint	0.6	0.012	14.7	0.294		
Seven Days						
Amber	1.6	0.032	62.2	1.244	72.0	1.440
Flint	0.2	0.004	10.8	0.216		
Ten Days						
Amber	1.0	0.020	57.9	1.158	71.6	1.432
Flint	0.0	0.000	4.3	0.086		
Fifteen Days						
Amber	0.3	0.006	56.0	1.120	71.2	1.432
Flint	...	.....	3.4	0.068		
Twenty Days						
Amber	0.0	0.000	55.3	1.106	70.9	1.418
Flint	...	.....	1.8	0.036		
Thirty Days						
Amber	...	.....	53.8	1.076	70.7	1.414
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	...	.....	44.2	0.884	69.8	1.396

TABLE 25

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 50 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 72.4 or 1.448 mcg./ml.						
One Day						
Amber	20.2	0.404	72.0	1.440	72.4	1.448
Flint	2.0	0.040	55.0	1.100		
Three Days						
Amber	8.0	0.160	71.5	1.430	72.4	1.448
Flint	0.9	0.008	29.3	0.586		
Five Days						
Amber	1.6	0.032	69.0	1.380	72.4	1.448
Flint	0.3	0.006	16.1	0.322		
Seven Days						
Amber	1.0	0.020	67.7	1.354	72.4	1.448
Flint	0.0	0.000	12.1	0.242		
Ten Days						
Amber	0.9	0.018	65.2	1.304	72.0	1.440
Flint	...	.....	5.0	0.100		
Fifteen Days						
Amber	0.1	0.002	62.4	1.248	71.8	1.436
Flint	...	.....	4.4	0.088		
Twenty Days						
Amber	0.0	0.000	58.5	1.170	71.6	1.432
Flint	...	.....	2.5	0.050		
Thirty Days						
Amber	...	.....	56.3	1.126	71.0	1.420
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	...	.....	47.3	0.946	69.4	1.388

TABLE 26

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN A SATURATED SOLUTION OF ETHYL AMINOBENZOATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 71.2 or 1.424 mcg./ml.						
One Day						
Amber	40.1	0.802	70.9	1.418	71.2	1.424
Flint	4.1	0.082	51.2	1.024		
Three Days						
Amber	27.2	0.544	70.4	1.408	71.2	1.424
Flint	3.2	0.064	43.3	0.866		
Five Days						
Amber	11.1	0.222	69.3	1.386	71.2	1.424
Flint	1.8	0.036	35.1	0.702		
Seven Days						
Amber	6.4	0.128	68.0	1.360	71.2	1.424
Flint	1.0	0.020	31.0	0.620		
Ten Days						
Amber	3.9	0.078	67.0	1.340	70.8	1.416
Flint	0.6	0.012	21.0	0.420		
Fifteen Days						
Amber	1.2	0.034	65.5	1.310	70.4	1.408
Flint	0.2	0.004	12.3	0.246		
Twenty Days						
Amber	0.5	0.010	63.5	1.270	70.0	1.400
Flint	0.0	0.000	5.8	0.116		
Thirty Days						
Amber	0.0	0.000	60.5	1.210	69.5	1.390
Flint	...	.....	2.9	0.058		
Sixty Days						
Amber	...	.....	55.2	1.044	68.7	1.374

TABLE 27

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 0.01 PER CENT QUININE BISULFATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 76.2 or 1.524 mcg./ml.						
One Day						
Amber	23.4	0.468	71.1	1.422	76.2	1.524
Flint	3.8	0.076	48.8	0.976		
Three Days						
Amber	19.2	0.384	70.1	1.402	76.2	1.524
Flint	2.2	0.044	25.2	0.504		
Five Days						
Amber	12.8	0.256	69.6	1.392	76.2	1.524
Flint	1.0	0.020	16.2	0.324		
Seven Days						
Amber	10.6	0.212	68.9	1.378	76.0	1.520
Flint	0.5	0.010	14.4	0.288		
Ten Days						
Amber	5.0	0.100	64.1	1.282	75.9	1.518
Flint	0.0	0.000	10.1	0.202		
Fifteen Days						
Amber	3.4	0.068	62.5	1.250	75.7	1.514
Flint	...	.....	8.0	0.160		
Twenty Days						
Amber	1.9	0.038	61.8	1.236	75.5	1.510
Flint	...	.....	6.8	0.136		
Thirty Days						
Amber	0.2	0.004	58.7	1.174	75.2	1.504
Flint	...	.....	3.2	0.064		
Sixty Days						
Amber	0.0	0.000	51.5	1.030	74.1	1.482

TABLE 28

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN A SATURATED SOLUTION OF BETA-METHYL UMBELLIFERONE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 69.7 or 1.394 mcg./ml.						
One Day						
Amber	29.1	0.582	64.8	1.296	69.7	1.394
Flint	3.2	0.064	43.8	0.876		
Three Days						
Amber	11.0	0.220	64.5	1.290	69.7	1.394
Flint	2.0	0.040	34.5	0.690		
Five Days						
Amber	6.5	0.130	64.0	1.280	69.7	1.394
Flint	1.1	0.022	25.2	0.504		
Seven Days						
Amber	4.8	0.096	63.0	1.260	69.7	1.394
Flint	0.4	0.008	20.0	0.400		
Ten Days						
Amber	1.5	0.030	59.3	1.186	69.5	1.390
Flint	0.0	0.000	9.5	0.190		
Fifteen Days						
Amber	0.8	0.016	57.0	1.140	68.9	1.378
Flint	...	.....	2.0	0.040		
Twenty Days						
Amber	0.2	0.004	54.1	1.082	68.9	1.378
Flint	...	.....	1.4	0.028		
Thirty Days						
Amber	0.0	0.000	50.2	1.004	68.5	1.370
Flint	...	.....	0.3	0.006		
Sixty Days						
Amber	...	.....	47.2	0.944	67.2	1.344

TABLE 29

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 1.0 PER CENT UREA IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 70.8 or 1.416 mcg./ml.						
One Day						
Amber	6.1	0.122	68.8	1.376	70.8	1.416
Flint	1.1	0.022	31.1	0.622		
Three Days						
Amber	5.8	0.116	67.8	1.356	70.8	1.416
Flint	0.8	0.016	11.0	0.220		
Five Days						
Amber	5.2	0.104	65.6	1.312	70.8	1.416
Flint	0.5	0.010	5.2	0.104		
Seven Days						
Amber	4.8	0.096	64.4	1.288	70.5	1.410
Flint	0.0	0.000	2.2	0.044		
Ten Days						
Amber	3.0	0.060	56.1	1.122	70.1	1.402
Flint	...	.....	1.0	0.020		
Fifteen Days						
Amber	1.6	0.032	46.8	0.936	69.5	1.390
Flint	...	.....	0.4	0.008		
Twenty Days						
Amber	0.5	0.010	32.1	0.642	69.0	1.380
Flint	...	.....	0.0	0.000		
Thirty Days						
Amber	0.0	0.000	27.2	0.544	68.6	1.372
Flint	...	.....	...	.....		
Sixty Days						
Amber	...	.....	15.1	0.302	66.2	1.324

TABLE 30

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 0.1 PER CENT TWEEN 80 IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 75.0 or 1.500 mcg./ml.						
One Day						
Amber	19.2	0.384	72.8	1.456	75.0	1.500
Flint	1.9	0.038	30.9	0.618		
Three Days						
Amber	3.9	0.078	71.3	1.426	75.0	1.500
Flint	0.6	0.012	12.2	0.244		
Five Days						
Amber	2.7	0.054	68.0	1.360	75.0	1.500
Flint	0.0	0.000	1.8	0.036		
Seven Days						
Amber	1.5	0.030	65.1	1.302	75.0	1.500
Flint	...	.....	1.5	0.030		
Ten Days						
Amber	0.9	0.018	60.5	1.210	74.8	1.496
Flint	...	.....	0.3	0.006		
Fifteen Days						
Amber	0.4	0.008	57.0	1.140	74.2	1.484
Flint	...	.....	0.0	0.000		
Twenty Days						
Amber	0.0	0.000	55.6	1.112	73.4	1.468
Flint	...	.....	...	.....		
Thirty Days						
Amber	...	.....	52.4	1.048	72.5	1.450
Flint	...	.....	...	.....		
Sixty Days						
Amber	...	.....	41.5	0.830	70.4	1.408

TABLE 31

THE STABILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM IN 0.5 PER CENT NIACIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 70.6 or 1.412 mcg./ml.						
One Day						
Amber	15.0	0.300	70.3	1.406	70.6	1.412
Flint	2.0	0.040	42.9	0.858		
Three Days						
Amber	4.9	0.098	68.6	1.372	70.6	1.412
Flint	1.1	0.022	14.4	0.288		
Five Days						
Amber	2.8	0.056	66.7	1.334	70.6	1.412
Flint	0.7	0.014	7.0	0.140		
Seven Days						
Amber	1.9	0.038	64.0	1.280	70.6	1.412
Flint	0.0	0.000	2.5	0.050		
Ten Days						
Amber	0.8	0.016	60.8	1.214	70.2	1.404
Flint	...	.....	1.5	0.030		
Fifteen Days						
Amber	0.1	0.002	58.1	1.162	69.7	1.394
Flint	...	.....	1.0	0.020		
Twenty Days						
Amber	0.0	0.000	56.4	1.128	69.2	1.384
Flint	...	.....	0.0	0.000		
Thirty Days						
Amber	...	.....	51.8	1.036	68.7	1.374
Flint	...	.....	...	.....		
Sixty Days						
Amber	...	.....	41.0	0.820	66.6	1.332

TABLE 32

THE SOLUBILITY OF RIBOFLAVIN-5'-PHOSPHATE SODIUM
IN SOME AQUEOUS SOLUTIONS AND OTHER SOLVENTS

	Average Lumetron Reading	Mg./Ml.
Distilled Water	4.9	35.71
0.9% Sodium Chloride	5.2	37.14
0.9% Potassium Chloride	5.2	37.14
1.0% Sodium Acid Phosphate	3.5	25.00
1.0% Potassium Acid Phosphate	3.0	21.43
1.0% Niacinamide	6.0	42.85
1.0% Urea	5.8	41.42
Propylene Glycol	8.0	5.71
Glycerin	12.5	8.93
Alcohol	4.0	0.08

Results with Flavaxin Soluble

Winthrop-Stearns' Flavaxin Soluble or riboflavin sodium-sodium tetraborate was selected for study because of its solubility and its popularity on the market.

The assay of this preparation was evaluated fluorophotometrically. It showed a 50.0 per cent riboflavin content. Accordingly, 0.5 Gm. of Flavaxin Soluble was equivalent to 1 Gm. of riboflavin.

One milligram of Flavaxin Soluble was added to each ml. of the solvents used for stability study. Since the lumetron was set for readings up to 2 mcg. per ml., each solution had to be further diluted by adding 3.4 ml. to a sufficient quantity of distilled water to make 1000 ml. Twenty-five milliliters of this dilution were used for fluorophotometric analysis.

The solubility of Winthrop-Stearns' product in some aqueous solutions and other solvents was determined. Further dilution with distilled water was found necessary with several solvents to be able to successfully determine the results on the lumetron.

TABLE 33

THE STABILITY OF FLAVAXIN SOLUBLE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 82.0 or 1.640 mcg./ml.						
One Day						
Amber	34.5	0.690	82.0	1.640	82.0	1.640
Flint	2.7	0.054	45.5	0.910		
Three Days						
Amber	14.0	0.280	79.8	1.596	82.0	1.640
Flint	1.1	0.022	28.2	0.564		
Five Days						
Amber	2.0	0.040	78.3	1.566	82.0	1.640
Flint	0.5	0.010	20.6	0.412		
Seven Days						
Amber	1.5	0.030	77.5	1.550	82.0	1.640
Flint	0.0	0.000	14.5	0.290		
Ten Days						
Amber	1.1	0.022	74.2	1.484	81.6	1.632
Flint	...	.....	8.9	0.178		
Fifteen Days						
Amber	0.8	0.016	70.0	1.400	81.0	1.620
Flint	...	.....	5.2	0.104		
Twenty Days						
Amber	0.0	0.000	65.3	1.306	80.2	1.604
Flint	...	.....	2.4	0.048		
Thirty Days						
Amber	...	.....	61.9	1.238	79.4	1.588
Flint	...	.....	1.2	0.024		
Sixty Days						
Amber	...	.....	46.8	0.936	77.2	1.544

TABLE 34

THE STABILITY OF FLAVAXIN SOLUBLE IN DISTILLED WATER BUFFERED AT pH 6 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 83.9 or 1.678 mcg./ml.						
One Day						
Amber	33.8	0.676	83.9	1.678	83.9	1.678
Flint	2.6	0.052	47.6	0.952		
Three Days						
Amber	15.2	0.302	81.5	1.630	83.9	1.678
Flint	1.3	0.026	29.0	0.580		
Five Days						
Amber	2.2	0.044	80.0	1.600	83.9	1.678
Flint	0.6	0.012	21.2	0.424		
Seven Days						
Amber	1.6	0.032	78.3	1.566	83.9	1.678
Flint	0.0	0.000	15.3	0.306		
Ten Days						
Amber	1.0	0.020	75.5	1.510	83.9	1.678
Flint	...	.....	9.4	0.188		
Fifteen Days						
Amber	0.6	0.012	72.3	1.446	82.8	1.656
Flint	...	.....	5.8	0.116		
Twenty Days						
Amber	0.0	0.000	68.5	1.370	82.1	1.642
Flint	...	.....	2.8	0.056		
Thirty Days						
Amber	...	.....	63.8	1.276	81.5	1.630
Flint	...	.....	1.5	0.030		
Sixty Days						
Amber	...	.....	48.9	0.978	79.0	1.580

TABLE 35

THE STABILITY OF FLAVAXIN SOLUBLE IN DISTILLED WATER BUFFERED AT pH 5 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 82.0 or 1.640 mcg./ml.						
One Day						
Amber	36.2	0.732	82.0	1.640	82.0	1.640
Flint	2.9	0.058	47.5	0.950		
Three Days						
Amber	16.3	0.326	81.4	1.628	82.0	1.640
Flint	1.5	0.030	28.4	0.568		
Five Days						
Amber	3.0	0.060	80.8	1.616	82.0	1.640
Flint	0.8	0.016	20.2	0.404		
Seven Days						
Amber	2.1	0.042	78.9	1.578	82.0	1.640
Flint	0.0	0.000	15.0	0.300		
Ten Days						
Amber	1.8	0.036	76.0	1.520	82.0	1.640
Flint	...	.....	8.9	0.178		
Fifteen Days						
Amber	0.9	0.018	72.8	1.456	81.2	1.624
Flint	...	.....	5.1	0.102		
Twenty Days						
Amber	0.2	0.004	68.8	1.376	80.7	1.614
Flint	...	.....	2.5	0.050		
Thirty Days						
Amber	0.0	0.000	63.5	1.270	79.8	1.596
Flint	...	.....	1.4	0.028		
Sixty Days						
Amber	...	.....	48.8	0.976	77.6	1.552

TABLE 36

THE STABILITY OF FLAVAXIN SOLUBLE IN DISTILLED WATER BUFFERED AT pH 4 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 84.5 or 1.690 mcg./ml.						
One Day						
Amber	39.6	0.792	84.5	1.690	84.5	1.690
Flint	3.3	0.066	52.5	1.050		
Three Days						
Amber	19.2	0.384	83.4	1.668	84.5	1.690
Flint	1.8	0.036	33.2	0.664		
Five Days						
Amber	3.8	0.076	82.3	1.646	84.5	1.690
Flint	1.0	0.020	24.4	0.488		
Seven Days						
Amber	1.8	0.036	81.7	1.634	84.5	1.690
Flint	0.0	0.000	17.2	0.344		
Ten Days						
Amber	1.0	0.020	80.2	1.604	84.0	1.680
Flint	...	.....	8.8	0.176		
Fifteen Days						
Amber	0.8	0.016	79.1	1.582	83.6	1.672
Flint	...	.....	5.3	0.106		
Twenty Days						
Amber	0.0	0.000	75.3	1.506	82.7	1.654
Flint	...	.....	2.6	0.052		
Thirty Days						
Amber	...	.....	70.1	1.402	82.1	1.642
Flint	...	.....	1.5	0.030		
Sixty Days						
Amber	...	.....	70.1	1.402	82.1	1.642

TABLE 37

THE STABILITY OF FLAVAXIN SOLUBLE IN 25 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 84.4 or 1.688 mcg./ml.						
One Day						
Amber	44.0	0.880	84.4	1.688	84.4	1.688
Flint	2.8	0.056	66.5	1.330		
Three Days						
Amber	29.5	0.590	82.5	1.650	84.4	1.688
Flint	1.5	0.030	41.5	0.830		
Five Days						
Amber	8.3	0.166	81.5	1.630	84.4	1.688
Flint	0.9	0.018	30.2	0.604		
Seven Days						
Amber	7.0	0.140	78.5	1.570	84.4	1.688
Flint	0.0	0.000	18.4	0.368		
Ten Days						
Amber	5.2	0.104	75.8	1.516	83.9	1.678
Flint	...	.....	11.8	0.236		
Fifteen Days						
Amber	3.0	0.060	73.9	1.578	83.3	1.666
Flint	...	.....	7.2	0.144		
Twenty Days						
Amber	1.2	0.024	70.1	1.402	82.8	1.656
Flint	...	.....	4.8	0.096		
Thirty Days						
Amber	0.7	0.014	65.0	1.300	82.3	1.646
Flint	...	.....	2.2	0.044		
Sixty Days						
Amber	0.0	0.000	49.2	0.984	80.0	1.600

TABLE 38

THE STABILITY OF FLAVAXIN SOLUBLE IN 50 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 83.9 or 1.678 mcg./ml.						
One Day						
Amber	58.3	1.166	83.9	1.678	83.9	1.678
Flint	3.1	0.062	75.0	1.500		
Three Days						
Amber	42.8	0.856	82.4	1.648	83.9	1.678
Flint	1.8	0.038	52.1	1.042		
Five Days						
Amber	7.6	0.154	81.1	1.622	83.9	1.678
Flint	1.0	0.020	37.0	0.740		
Seven Days						
Amber	6.5	0.130	79.1	1.582	83.9	1.678
Flint	0.0	0.000	19.2	0.384		
Ten Days						
Amber	4.5	0.090	77.5	1.550	83.5	1.670
Flint	...	.....	12.4	0.248		
Fifteen Days						
Amber	3.5	0.070	75.5	1.510	82.9	1.658
Flint	...	.....	8.1	0.162		
Twenty Days						
Amber	1.8	0.036	71.2	1.424	82.3	1.646
Flint	...	.....	4.5	0.090		
Thirty Days						
Amber	0.8	0.016	66.3	1.326	81.9	1.638
Flint	...	.....	2.2	0.044		
Sixty Days						
Amber	0.0	0.000	50.2	1.004	80.0	1.600

TABLE 39

THE STABILITY OF FLAVAXIN SOLUBLE IN 25 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 83.9 or 1.678 mcg./ml.						
One Day						
Amber	37.5	0.750	83.9	1.678	83.9	1.678
Flint	2.5	0.050	63.3	1.266		
Three Days						
Amber	21.5	0.430	82.5	1.650	83.9	1.678
Flint	1.4	0.028	32.7	0.654		
Five Days						
Amber	4.5	0.090	81.7	1.634	83.9	1.678
Flint	0.8	0.016	20.5	0.410		
Seven Days						
Amber	3.7	0.074	80.8	1.616	83.9	1.678
Flint	0.0	0.000	11.7	0.234		
Ten Days						
Amber	3.0	0.060	77.3	1.546	83.3	1.666
Flint	...	.....	7.5	0.150		
Fifteen Days						
Amber	1.5	0.030	72.3	1.446	82.8	1.656
Flint	...	.....	4.5	0.090		
Twenty Days						
Amber	0.3	0.006	67.2	1.344	82.0	1.640
Flint	...	.....	1.8	0.036		
Thirty Days						
Amber	0.0	0.000	63.3	1.266	81.7	1.634
Flint	...	.....	0.3	0.006		
Sixty Days						
Amber	...	.....	48.2	0.964	79.6	1.592

TABLE 40

THE STABILITY OF FLAVAXIN SOLUBLE IN 50 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 85.0 or 1.700 mcg./ml.						
One Day						
Amber	45.0	0.900	85.0	1.700	85.0	1.700
Flint	3.0	0.060	69.9	1.398		
Three Days						
Amber	25.5	0.510	83.9	1.678	85.0	1.700
Flint	1.8	0.036	44.9	0.898		
Five Days						
Amber	5.3	0.106	82.2	1.644	85.0	1.700
Flint	1.0	0.002	29.6	0.592		
Seven Days						
Amber	3.9	0.078	81.4	1.628	85.0	1.700
Flint	0.0	0.000	16.9	0.338		
Ten Days						
Amber	2.8	0.056	79.7	1.594	85.0	1.700
Flint	...	.....	8.9	0.178		
Fifteen Days						
Amber	2.0	0.040	76.4	1.524	84.6	1.692
Flint	...	.....	5.0	0.100		
Twenty Days						
Amber	0.8	0.016	72.3	1.446	83.8	1.676
Flint	...	.....	2.1	0.042		
Thirty Days						
Amber	0.0	0.000	67.0	1.340	83.0	1.660
Flint	...	.....	0.8	0.016		
Sixty Days						
Amber	...	.....	50.2	1.004	81.1	1.622

TABLE 41

THE STABILITY OF FLAVAXIN SOLUBLE IN A SATURATED SOLUTION OF ETHYL AMINOBENZOATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 85.6 or 1.712 mcg./ml.						
One Day						
Amber	40.2	0.804	85.6	1.712	85.6	1.712
Flint	3.2	0.064	65.0	1.300		
Three Days						
Amber	32.2	0.644	84.2	1.684	85.6	1.712
Flint	1.9	0.038	52.2	1.044		
Five Days						
Amber	12.5	0.250	83.7	1.674	85.6	1.712
Flint	1.1	0.022	47.0	0.940		
Seven Days						
Amber	7.4	0.148	82.4	1.648	85.6	1.712
Flint	0.0	0.000	40.0	0.800		
Ten Days						
Amber	4.2	0.084	80.0	1.600	85.6	1.712
Flint	...	.....	31.9	0.638		
Fifteen Days						
Amber	1.8	0.036	79.0	1.580	85.6	1.712
Flint	...	.....	19.2	0.384		
Twenty Days						
Amber	1.0	0.020	77.6	1.552	85.0	1.700
Flint	...	.....	8.8	0.176		
Thirty Days						
Amber	0.6	0.012	74.3	1.486	84.8	1.696
Flint	...	.....	3.8	0.076		
Sixty Days						
Amber	0.0	0.000	65.8	1.376	84.4	1.688

TABLE 42

THE STABILITY OF FLAVAXIN SOLUBLE IN 0.01 PER CENT QUININE BISULFATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 83.7 or 1.674 mcg./ml.						
One Day						
Amber	51.7	1.034	83.7	1.674	83.7	1.674
Flint	3.0	0.060	57.3	1.146		
Three Days						
Amber	38.0	0.760	83.1	1.662	83.7	1.674
Flint	1.8	0.036	48.8	0.976		
Five Days						
Amber	14.1	0.282	82.2	1.644	83.7	1.674
Flint	0.9	0.018	37.3	0.746		
Seven Days						
Amber	4.3	0.086	81.5	1.630	83.7	1.674
Flint	0.0	0.000	24.0	0.480		
Ten Days						
Amber	2.4	0.048	80.7	1.614	83.5	1.670
Flint	...	.....	17.2	0.344		
Fifteen Days						
Amber	1.1	0.022	79.3	1.586	83.1	1.662
Flint	...	.....	12.1	0.242		
Twenty Days						
Amber	0.5	0.010	77.2	1.544	82.8	1.656
Flint	...	.....	7.6	0.152		
Thirty Days						
Amber	0.0	0.000	72.2	1.444	82.4	1.648
Flint	...	.....	2.7	0.054		
Sixty Days						
Amber	...	.....	59.3	1.186	81.2	1.624

TABLE 43

THE STABILITY OF FLAVAXIN SOLUBLE IN A SATURATED SOLUTION OF BETA-METHYL UMBELLIFERONE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 85.5 or 1.710 mcg./ml.						
One Day						
Amber	45.3	0.906	85.5	1.710	85.5	1.710
Flint	2.8	0.056	54.5	1.090		
Three Days						
Amber	29.3	0.586	84.8	1.696	85.5	1.710
Flint	1.2	0.024	39.1	0.782		
Five Days						
Amber	12.1	0.242	83.6	1.672	85.5	1.710
Flint	0.7	0.014	28.9	0.578		
Seven Days						
Amber	5.4	0.108	81.8	1.636	85.5	1.710
Flint	0.0	0.000	17.8	0.356		
Ten Days						
Amber	3.1	0.062	80.1	1.602	85.2	1.704
Flint	...	.....	8.2	0.164		
Fifteen Days						
Amber	1.5	0.030	79.2	1.584	84.8	1.696
Flint	...	.....	4.9	0.198		
Twenty Days						
Amber	0.8	0.016	78.3	1.566	84.5	1.690
Flint	...	.....	2.1	0.042		
Thirty Days						
Amber	0.0	0.000	73.2	1.464	84.2	1.684
Flint	...	.....	0.8	0.016		
Sixty Days						
Amber	...	.....	60.4	1.208	83.0	1.660

TABLE 44

THE STABILITY OF FLAVAXIN SOLUBLE IN 0.1 PER CENT TWEEN 80 IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 84.5 or 1.690 mcg./ml.						
One Day						
Amber	36.2	0.724	84.5	1.690	84.5	1.690
Flint	3.0	0.060	48.0	0.960		
Three Days						
Amber	16.2	0.324	82.0	1.640	84.5	1.690
Flint	1.8	0.036	30.4	0.608		
Five Days						
Amber	1.8	0.036	80.5	1.610	84.5	1.690
Flint	0.9	0.018	23.0	0.460		
Seven Days						
Amber	1.0	0.020	79.5	1.590	84.5	1.690
Flint	0.0	0.000	12.9	0.258		
Ten Days						
Amber	0.9	0.018	76.3	1.526	84.0	1.680
Flint	...	.....	9.1	0.182		
Fifteen Days						
Amber	0.3	0.006	71.5	1.430	83.7	1.674
Flint	...	.....	4.9	0.098		
Twenty Days						
Amber	0.0	0.000	66.0	1.320	82.2	1.644
Flint	...	.....	2.4	0.048		
Thirty Days						
Amber	...	.....	60.0	1.200	81.0	1.622
Flint	...	.....	1.1	0.022		
Sixty Days						
Amber	...	.....	47.0	0.940	79.7	1.594

TABLE 45

THE STABILITY OF FLAVAXIN SOLUBLE IN 1.0 PER CENT UREA IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 84.5 or 1.690 mcg./ml.						
One Day						
Amber	36.3	0.726	84.5	1.690	84.5	1.690
Flint	2.5	0.050	69.5	1.390		
Three Days						
Amber	10.0	0.200	81.2	1.624	84.5	1.690
Flint	1.8	0.036	37.5	0.750		
Five Days						
Amber	7.5	0.150	79.5	1.590	84.5	1.690
Flint	0.9	0.018	15.8	0.316		
Seven Days						
Amber	4.6	0.092	75.8	1.516	84.5	1.690
Flint	0.0	0.000	9.6	0.192		
Ten Days						
Amber	3.8	0.076	64.6	1.292	84.0	1.680
Flint	...	.....	5.5	0.110		
Fifteen Days						
Amber	1.5	0.030	58.2	1.164	83.7	1.674
Flint	...	.....	4.5	0.090		
Twenty Days						
Amber	0.9	0.018	50.0	1.000	82.9	1.658
Flint	...	.....	2.8	0.056		
Thirty Days						
Amber	0.0	0.000	38.2	0.764	82.2	1.644
Flint	...	.....	1.2	0.024		
Sixty Days						
Amber	...	.....	27.8	0.556	80.0	1.600

TABLE 46

THE STABILITY OF FLAVAXIN SOLUBLE IN 0.5 PER CENT NIACIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 84.6 or 1.692 mcg./ml.						
One Day						
Amber	39.5	0.790	84.6	1.692	84.6	1.692
Flint	3.2	0.064	55.2	1.104		
Three Days						
Amber	19.0	0.380	83.3	1.666	84.6	1.692
Flint	1.7	0.034	30.1	0.602		
Five Days						
Amber	3.5	0.070	82.1	1.642	84.6	1.692
Flint	1.0	0.020	25.0	0.500		
Seven Days						
Amber	1.6	0.032	81.3	1.626	84.6	1.692
Flint	0.0	0.000	16.1	0.322		
Ten Days						
Amber	0.9	0.018	80.6	1.612	84.2	1.684
Flint	...	.....	7.8	0.156		
Fifteen Days						
Amber	0.7	0.014	79.2	1.584	83.8	1.676
Flint	...	.....	4.9	0.098		
Twenty Days						
Amber	0.0	0.000	75.4	1.508	83.2	1.664
Flint	...	.....	3.0	0.060		
Thirty Days						
Amber	...	.....	70.2	1.404	82.3	1.646
Flint	...	.....	1.8	0.036		
Sixty Days						
Amber	...	.....	54.6	1.092	80.2	1.604

TABLE 47

THE SOLUBILITY OF FLAVAXIN SOLUBLE IN SOME AQUEOUS SOLUTIONS AND OTHER SOLVENTS

	Average Lumetron Reading	Mg./Ml.
Distilled Water	99.5	3.98
0.9% Sodium Chloride	99.0	3.96
0.9% Potassium Chloride	99.0	3.96
1.0% Sodium Acid Phosphate	54.2	2.16
1.0% Potassium Acid Phosphate	48.0	1.92
1.0% Niacinamide	10.5	10.50
1.0% Urea	4.2	4.20
Propylene Glycol	13.0	13.00
Glycerin	21.6	21.60
Alcohol	22.5	0.45

Results with a Pyruvic Acid Derivative of Riboflavin

The pyruvic acid derivative of riboflavin was prepared according to the procedure listed under the preparation of riboflavin derivatives. This derivative was a yellowish-orange crystalline powder, differing in color from pure riboflavin by being somewhat lighter. It was hygroscopic. When dry, it was not appreciably affected by diffused light. The melting point was between 168-172° C.

Assay of the pyruvic acid derivative by the fluorophotometric procedure showed a 70.0 per cent riboflavin content. Accordingly, 0.7 Gm. of riboflavin was equivalent to 1 Gm. of the pyruvic acid derivative.

One milligram of the derivative was added to each ml. of the solvents used for stability study. Since the lumetron was set for determinations up to 2 mcg. per ml., each solution had to be further diluted by adding 2.5 ml. to a sufficient quantity of distilled water to make 1000 ml. Twenty-five milliliters of this dilution were used for fluorophotometric analysis.

The solubility in the various solvents mentioned previously was evaluated for the pyruvic acid derivative.

TABLE 48

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 86.2 or 1.724 mcg./ml.						
One Day						
Amber	50.1	1.002	85.0	1.700	86.2	1.724
Flint	1.2	0.024	50.8	1.016		
Three Days						
Amber	20.4	0.408	82.3	1.646	86.2	1.724
Flint	0.8	0.016	34.0	0.680		
Five Days						
Amber	7.2	0.144	81.1	1.622	86.2	1.724
Flint	0.3	0.006	18.5	0.370		
Seven Days						
Amber	3.8	0.076	79.4	1.588	86.2	1.724
Flint	0.0	0.000	8.0	0.160		
Ten Days						
Amber	1.5	0.030	79.5	1.570	86.0	1.720
Flint	...	.....	5.5	0.110		
Fifteen Days						
Amber	1.3	0.026	76.8	1.536	85.8	1.716
Flint	...	.....	3.9	0.078		
Twenty Days						
Amber	1.0	0.020	70.1	1.402	85.4	1.708
Flint	...	.....	2.0	0.040		
Thirty Days						
Amber	0.4	0.008	64.0	1.280	85.0	1.700
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	46.2	0.924	83.4	1.668

TABLE 49

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 6 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 86.2 or 1.724 mcg./ml.						
One Day						
Amber	52.1	1.042	84.8	1.696	86.2	1.724
Flint	1.5	0.030	47.5	0.950		
Three Days						
Amber	23.4	0.468	82.0	1.640	86.2	1.724
Flint	0.9	0.018	28.4	0.568		
Five Days						
Amber	9.1	0.182	81.3	1.626	86.2	1.724
Flint	0.4	0.008	14.5	0.290		
Seven Days						
Amber	4.2	0.084	79.8	1.596	86.2	1.724
Flint	0.0	0.000	6.5	0.130		
Ten Days						
Amber	2.8	0.056	78.5	1.570	86.0	1.720
Flint	...	.....	4.3	0.086		
Fifteen Days						
Amber	1.5	0.030	74.9	1.498	85.7	1.714
Flint	...	.....	3.5	0.070		
Twenty Days						
Amber	1.1	0.022	68.2	1.364	85.2	1.704
Flint	...	.....	1.9	0.038		
Thirty Days						
Amber	0.7	0.014	63.8	1.276	84.8	1.696
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	45.8	0.896	83.2	1.664

TABLE 50

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 5 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 82.3 or 1.646 mcg./ml.						
One Day						
Amber	55.4	1.108	80.8	1.616	82.3	1.646
Flint	1.5	0.030	50.2	1.004		
Three Days						
Amber	25.2	0.504	79.0	1.580	82.3	1.646
Flint	0.8	0.016	30.4	0.608		
Five Days						
Amber	11.3	0.226	78.2	1.564	82.3	1.646
Flint	0.3	0.006	16.0	0.320		
Seven Days						
Amber	5.8	0.116	75.7	1.514	82.3	1.646
Flint	0.0	0.000	4.2	0.084		
Ten Days						
Amber	3.0	0.060	74.2	1.484	82.1	1.642
Flint	...	.....	3.1	0.062		
Fifteen Days						
Amber	1.9	0.038	72.4	1.448	81.8	1.636
Flint	...	.....	1.2	0.024		
Twenty Days						
Amber	1.1	0.022	70.2	1.404	81.0	1.620
Flint	...	.....	1.0	0.020		
Thirty Days						
Amber	0.5	0.010	65.4	1.308	80.7	1.614
Flint	...	.....	0.3	0.006		
Sixty Days						
Amber	0.0	0.000	45.8	0.916	78.8	1.576

TABLE 51

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 4 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 80.1 or 1.602						
One Day						
Amber	56.2	1.124	78.9	1.578	80.1	1.602
Flint	1.4	0.028	49.8	0.996		
Three Days						
Amber	25.3	0.506	77.4	1.548	80.1	1.602
Flint	0.7	0.014	29.5	0.590		
Five Days						
Amber	10.4	0.208	76.8	1.536	80.1	1.602
Flint	0.2	0.004	15.4	0.308		
Seven Days						
Amber	6.0	0.120	76.0	1.520	80.1	1.602
Flint	0.0	0.000	5.0	0.100		
Ten Days						
Amber	2.9	0.058	75.4	1.508	80.1	1.602
Flint	...	.....	3.8	0.076		
Fifteen Days						
Amber	1.8	0.036	74.6	1.492	79.7	1.594
Flint	...	.....	1.9	0.038		
Twenty Days						
Amber	1.0	0.020	72.0	1.458	79.1	1.582
Flint	...	.....	1.5	0.030		
Thirty Days						
Amber	0.4	0.008	66.1	1.322	78.3	1.566
Flint	...	.....	0.2	0.004		
Sixty Days						
Amber	0.0	0.000	46.6	0.932	77.2	1.544

TABLE 52

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 25 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 86.2 or 1.724 mcg./ml.						
One Day						
Amber	61.3	1.226	86.2	1.724	86.2	1.724
Flint	1.5	0.030	82.1	1.642		
Three Days						
Amber	21.1	0.422	85.8	1.716	86.2	1.724
Flint	0.8	0.016	62.2	1.244		
Five Days						
Amber	10.3	0.206	84.2	1.684	86.2	1.724
Flint	0.1	0.002	47.5	0.950		
Seven Days						
Amber	7.0	0.140	83.6	1.672	86.2	1.724
Flint	0.0	0.000	32.8	0.656		
Ten Days						
Amber	3.3	0.066	81.5	1.630	86.2	1.724
Flint	...	.....	22.8	0.456		
Fifteen Days						
Amber	2.9	0.058	78.5	1.570	85.8	1.716
Flint	...	.....	14.3	0.286		
Twenty Days						
Amber	2.2	0.044	76.1	1.522	85.4	1.708
Flint	...	.....	8.5	0.170		
Thirty Days						
Amber	0.9	0.018	66.5	1.330	84.0	1.680
Flint	...	.....	2.4	0.048		
Sixty Days						
Amber	0.0	0.000	49.5	0.990	82.4	1.648

TABLE 53

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 50 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 89.5 or 1.790 mcg./ml.						
One Day						
Amber	68.9	1.378	89.5	1.790	89.5	1.790
Flint	1.9	0.038	83.3	1.666		
Three Days						
Amber	33.5	0.670	88.8	1.776	89.5	1.790
Flint	0.9	0.018	65.1	1.302		
Five Days						
Amber	15.5	0.310	88.0	1.760	89.5	1.790
Flint	0.2	0.004	50.7	1.014		
Seven Days						
Amber	9.2	0.184	87.1	1.742	89.5	1.790
Flint	0.0	0.000	34.2	0.684		
Ten Days						
Amber	3.5	0.070	85.2	1.704	89.5	1.790
Flint	...	.....	20.3	0.406		
Fifteen Days						
Amber	3.0	0.060	82.5	1.650	89.0	1.780
Flint	...	.....	10.2	0.204		
Twenty Days						
Amber	2.4	0.048	79.6	1.592	88.6	1.772
Flint	...	.....	4.6	0.092		
Thirty Days						
Amber	1.4	0.028	71.5	1.430	87.8	1.756
Flint	...	.....	2.8	0.056		
Sixty Days						
Amber	0.0	0.000	65.0	1.300	85.9	1.718

TABLE 54

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 25 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 88.4 or 1.768 mcg./ml.						
One Day						
Amber	56.0	1.120	88.4	1.768	88.4	1.768
Flint	1.7	0.034	78.2	1.564		
Three Days						
Amber	20.6	0.412	87.3	1.746	88.4	1.768
Flint	0.6	0.012	54.6	1.092		
Five Days						
Amber	10.0	0.200	86.1	1.722	88.4	1.768
Flint	0.1	0.002	40.3	0.806		
Seven Days						
Amber	6.9	0.138	85.2	1.704	88.4	1.768
Flint	0.0	0.000	27.4	0.548		
Ten Days						
Amber	2.9	0.058	83.7	1.674	88.0	1.760
Flint	...	.....	18.0	0.360		
Fifteen Days						
Amber	2.5	0.050	80.5	1.610	87.6	1.752
Flint	...	.....	10.6	0.212		
Twenty Days						
Amber	1.9	0.038	76.6	1.532	86.9	1.738
Flint	...	.....	6.4	0.128		
Thirty Days						
Amber	0.5	0.010	67.8	1.356	86.2	1.724
Flint	...	.....	3.2	0.064		
Sixty Days						
Amber	0.0	0.000	62.3	1.246	84.0	1.680

TABLE 55

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 50 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 89.8 or 1.796 mcg./ml.						
One Day						
Amber	62.3	1.246	89.0	1.780	89.8	1.796
Flint	1.6	0.032	82.6	1.652		
Three Days						
Amber	29.0	0.580	88.8	1.776	89.8	1.796
Flint	0.8	0.016	64.9	1.298		
Five Days						
Amber	12.4	0.248	87.8	1.756	89.8	1.796
Flint	0.2	0.004	53.8	1.076		
Seven Days						
Amber	7.0	0.140	86.0	1.720	89.8	1.796
Flint	0.0	0.000	38.6	0.772		
Ten Days						
Amber	4.2	0.084	84.7	1.694	89.2	1.784
Flint	...	.....	25.8	0.516		
Fifteen Days						
Amber	1.8	0.036	81.0	1.620	88.7	1.774
Flint	...	.....	16.2	0.324		
Twenty Days						
Amber	1.5	0.030	78.6	1.572	88.1	1.762
Flint	...	.....	9.7	0.194		
Thirty Days						
Amber	0.9	0.018	71.0	1.420	87.2	1.744
Flint	...	.....	4.3	0.086		
Sixty Days						
Amber	0.0	0.000	64.8	1.296	86.8	1.736

TABLE 56

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN A SATURATED SOLUTION OF ETHYL AMINOBENZOATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 83.5 or 1.670 mcg./ml.						
One Day						
Amber	69.7	1.394	82.5	1.650	83.5	1.670
Flint	2.5	0.030	79.8	1.596		
Three Days						
Amber	40.9	0.818	81.8	1.636	83.5	1.670
Flint	1.3	0.026	58.4	1.168		
Five Days						
Amber	29.8	0.596	81.0	1.620	83.5	1.670
Flint	0.9	0.018	45.3	0.906		
Seven Days						
Amber	22.2	0.444	80.2	1.604	83.5	1.670
Flint	0.5	0.010	36.4	0.728		
Ten Days						
Amber	17.3	0.346	79.5	1.590	83.5	1.670
Flint	0.0	0.000	22.9	0.458		
Fifteen Days						
Amber	7.4	0.148	77.7	1.554	83.1	1.662
Flint	...	.....	18.6	0.372		
Twenty Days						
Amber	2.8	0.056	75.6	1.516	82.8	1.656
Flint	...	.....	11.0	0.220		
Thirty Days						
Amber	1.7	0.034	72.8	1.456	82.5	1.650
Flint	...	.....	6.1	0.122		
Sixty Days						
Amber	0.0	0.000	69.5	1.390	81.7	1.634

TABLE 57

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 0.01 PER CENT QUININE BISULFATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 83.8 or 1.676 mcg./ml.						
One Day						
Amber	55.3	1.106	81.3	1.626	83.8	1.676
Flint	1.9	0.038	76.3	1.526		
Three Days						
Amber	35.0	0.680	80.0	1.600	83.8	1.676
Flint	1.0	0.020	57.3	1.146		
Five Days						
Amber	20.0	0.400	79.2	1.584	83.8	1.676
Flint	0.8	0.016	40.5	0.810		
Seven Days						
Amber	12.4	0.248	78.0	1.560	83.8	1.676
Flint	0.2	0.004	33.4	0.668		
Ten Days						
Amber	7.6	0.152	77.5	1.550	83.8	1.676
Flint	0.0	0.000	19.5	0.390		
Fifteen Days						
Amber	5.1	0.102	74.1	1.482	83.5	1.670
Flint	...	.....	15.3	0.306		
Twenty Days						
Amber	3.8	0.076	71.8	1.436	83.1	1.662
Flint	...	.....	9.5	0.190		
Thirty Days						
Amber	1.0	0.020	68.9	1.378	82.8	1.656
Flint	...	.....	4.2	0.084		
Sixty Days						
Amber	0.0	0.000	62.3	1.246	81.7	1.634

TABLE 58

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN A SATURATED SOLUTION OF BETA-METHYL UMBELLIFERONE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 81.3 or 1.626 mcg./ml.						
One Day						
Amber	63.0	1.260	80.5	1.610	81.3	1.626
Flint	1.8	0.036	75.5	1.510		
Three Days						
Amber	36.2	0.724	79.6	1.592	81.3	1.626
Flint	1.0	0.020	54.3	1.086		
Five Days						
Amber	22.2	0.444	78.2	1.564	81.3	1.626
Flint	0.7	0.014	39.4	0.788		
Seven Days						
Amber	14.3	0.286	77.6	1.552	81.3	1.626
Flint	0.1	0.002	28.3	0.566		
Ten Days						
Amber	10.5	0.210	77.0	1.540	81.3	1.626
Flint	0.0	0.000	19.8	0.396		
Fifteen Days						
Amber	4.2	0.084	76.1	1.522	81.0	1.620
Flint	...	.....	13.2	0.264		
Twenty Days						
Amber	3.5	0.070	75.2	1.504	80.6	1.612
Flint	...	.....	3.3	0.066		
Thirty Days						
Amber	1.1	0.022	70.2	1.404	79.0	1.580
Flint	...	.....	1.2	0.024		
Sixty Days						
Amber	0.0	0.000	57.2	1.144	78.8	1.576

TABLE 59

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 1.0 PER CENT UREA IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 85.6 or 1.712 mcg./ml.						
One Day						
Amber	36.2	0.724	84.5	1.690	85.6	1.712
Flint	1.0	0.020	71.9	1.438		
Three Days						
Amber	12.3	0.246	82.7	1.654	85.6	1.712
Flint	0.7	0.014	32.0	0.640		
Five Days						
Amber	8.2	0.164	81.9	1.638	85.6	1.712
Flint	0.3	0.006	24.4	0.488		
Seven Days						
Amber	4.6	0.092	76.3	1.526	85.6	1.712
Flint	0.0	0.000	10.1	0.202		
Ten Days						
Amber	4.0	0.080	67.3	1.346	85.6	1.712
Flint	...	.....	4.2	0.084		
Fifteen Days						
Amber	2.3	0.046	54.0	1.080	85.0	1.700
Flint	...	.....	1.9	0.038		
Twenty Days						
Amber	2.1	0.042	41.8	0.836	84.5	1.690
Flint	...	.....	0.6	0.012		
Thirty Days						
Amber	0.9	0.018	27.2	0.544	83.5	1.670
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	19.4	0.388	81.2	1.624

TABLE 60

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 0.1 PER CENT TWEEN 80 IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 85.8 or 1.716 mcg./ml.						
One Day						
Amber	49.1	0.982	85.0	1.700	85.8	1.716
Flint	1.0	0.020	48.2	0.964		
Three Days						
Amber	19.7	0.394	81.2	1.624	85.8	1.716
Flint	0.7	0.014	33.3	0.666		
Five Days						
Amber	6.0	0.120	80.1	1.602	85.8	1.716
Flint	0.2	0.004	16.8	0.336		
Seven Days						
Amber	2.8	0.056	78.3	1.566	85.8	1.716
Flint	0.0	0.000	9.2	0.182		
Ten Days						
Amber	1.4	0.028	76.8	1.536	85.8	1.716
Flint	...	.....	5.5	0.110		
Fifteen Days						
Amber	1.1	0.022	75.1	1.502	85.2	1.704
Flint	...	.....	3.4	0.068		
Twenty Days						
Amber	0.7	0.014	71.0	1.420	84.8	1.696
Flint	...	.....	1.8	0.036		
Thirty Days						
Amber	0.2	0.004	65.5	1.310	84.2	1.684
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	45.3	0.906	82.7	1.654

TABLE 61

THE STABILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN 0.5 PER CENT NIACIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 82.5 or 1.650 mcg./ml.						
One Day						
Amber	58.4	1.168	81.1	1.622	82.5	1.650
Flint	1.4	0.028	51.8	1.036		
Three Days						
Amber	26.3	0.526	79.2	1.584	82.5	1.650
Flint	0.8	0.016	32.4	0.268		
Five Days						
Amber	11.0	0.220	78.6	1.572	82.5	1.650
Flint	0.1	0.002	18.3	0.366		
Seven Days						
Amber	5.2	0.104	77.9	1.558	82.5	1.650
Flint	0.0	0.000	7.6	0.152		
Ten Days						
Amber	3.0	0.060	76.8	1.536	82.0	1.640
Flint	...	.....	4.0	0.080		
Fifteen Days						
Amber	1.9	0.038	76.3	1.526	81.6	1.632
Flint	...	.....	3.5	0.070		
Twenty Days						
Amber	1.1	0.022	74.1	1.482	81.1	1.622
Flint	...	.....	2.0	0.040		
Thirty Days						
Amber	0.5	0.010	69.0	1.380	80.5	1.610
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	48.0	0.960	78.4	1.568

TABLE 62

THE SOLUBILITY OF A PYRUVIC ACID DERIVATIVE OF RIBOFLAVIN IN SOME AQUEOUS SOLUTIONS AND OTHER SOLVENTS

	Average Lumetron Reading	Mg./Ml.
Distilled Water	70.0	2.00
0.9% Sodium Chloride	74.0	2.11
0.9% Potassium Chloride	79.5	2.27
1.0% Sodium Acid Phosphate	75.0	2.14
1.0% Potassium Acid Phosphate	75.0	2.14
1.0% Niacinamide	95.0	2.71
1.0% Urea	76.1	2.17
Propylene Glycol	78.3	2.23
Glycerin	63.3	1.80
Alcohol	26.5	0.74

Results with a Levulinic Acid Derivative of Riboflavin

The levulinic acid derivative was prepared according to the general procedure employed in this investigation for making riboflavin derivatives. This derivative was a yellow crystalline powder. It was hygroscopic and melted between 224-228° C.

Assay of the levulinic acid derivative employing fluorophotometric procedure yielded an 80.2 per cent riboflavin content. Thus, 0.802 Gm. of riboflavin was equivalent to 1 Gm. of the derivative.

One milligram of the derivative was added to each ml. of the solvents previously mentioned for stability study. Since the lumetron was set for determinations up to 2 mcg. per ml., each solution had to be further diluted by adding 2.2 ml. of the vitamin solution to sufficient distilled water to make 1000 ml. Twenty-five milliliters of this dilution were used for fluorophotometric analysis.

Solubility studies were evaluated for the levulinic acid derivative.

Table 63

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 88.6 or 1.772 mcg./ml.						
One Day						
Amber	53.4	1.068	86.5	1.730	88.6	1.772
Flint	2.8	0.056	59.2	1.184		
Three Days						
Amber	26.0	0.520	84.8	1.696	88.6	1.772
Flint	1.4	0.028	35.5	0.710		
Five Days						
Amber	12.8	0.256	83.1	1.662	88.6	1.772
Flint	0.4	0.008	16.6	0.332		
Seven Days						
Amber	4.5	0.090	82.0	1.640	88.6	1.772
Flint	0.0	0.000	9.0	0.180		
Ten Days						
Amber	2.9	0.058	78.0	1.560	88.2	1.764
Flint	...	.....	4.8	0.090		
Fifteen Days						
Amber	1.5	0.030	77.0	1.540	87.8	1.756
Flint	...	.....	3.3	0.066		
Twenty Days						
Amber	1.1	0.022	71.6	1.432	87.6	1.752
Flint	...	.....	1.7	0.034		
Thirty Days						
Amber	0.3	0.006	68.4	1.368	87.2	1.744
Flint	...	.....	0.8	0.016		
Sixty Days						
Amber	0.0	0.000	49.5	0.990	85.7	1.714

TABLE 64

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 6 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 88.4 or 1.768 mcg./ml.						
One Day						
Amber	54.6	1.092	86.0	1.720	88.4	1.768
Flint	2.7	0.054	48.2	0.964		
Three Days						
Amber	25.0	0.500	84.4	1.688	88.4	1.768
Flint	1.5	0.030	29.0	0.580		
Five Days						
Amber	13.0	0.260	83.0	1.660	88.4	1.768
Flint	0.5	0.010	15.0	0.300		
Seven Days						
Amber	4.8	0.096	81.8	1.636	88.4	1.768
Flint	0.0	0.000	6.8	0.136		
Ten Days						
Amber	3.2	0.064	78.2	1.564	88.4	1.768
Flint	...	.....	4.8	0.096		
Fifteen Days						
Amber	1.5	0.030	76.6	1.532	87.9	1.758
Flint	...	.....	3.9	0.078		
Twenty Days						
Amber	1.3	0.026	70.5	1.410	87.5	1.750
Flint	...	.....	2.0	0.040		
Thirty Days						
Amber	0.4	0.008	67.9	1.358	86.8	1.736
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	50.6	1.012	85.3	1.706

TABLE 65

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 5 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 86.4 or 1.728 mcg./ml.						
One Day						
Amber	54.3	1.086	84.2	1.684	86.4	1.728
Flint	2.6	0.052	47.2	0.944		
Three Days						
Amber	24.8	0.496	82.3	1.646	86.4	1.728
Flint	1.5	0.030	28.5	0.570		
Five Days						
Amber	12.6	0.252	81.1	1.622	86.4	1.728
Flint	0.4	0.008	15.4	0.308		
Seven Days						
Amber	4.5	0.090	79.8	1.596	86.4	1.728
Flint	0.0	0.000	7.0	0.140		
Ten Days						
Amber	3.0	0.060	76.2	1.524	86.0	1.720
Flint	...	.....	4.5	0.090		
Fifteen Days						
Amber	1.6	0.032	74.9	1.498	85.7	1.714
Flint	...	.....	3.6	0.072		
Twenty Days						
Amber	1.0	0.020	73.4	1.468	85.4	1.708
Flint	...	.....	2.0	0.040		
Thirty Days						
Amber	0.3	0.006	69.0	1.380	85.0	1.700
Flint	...	.....	0.3	0.006		
Sixty Days						
Amber	0.0	0.000	51.8	1.036	83.8	1.676

TABLE 66

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN DISTILLED WATER BUFFERED AT pH 4 STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 86.6 or 1.732 mcg./ml.						
One Day						
Amber	55.8	1.116	84.8	1.696	86.6	1.732
Flint	2.8	0.056	49.1	0.982		
Three Days						
Amber	27.3	0.546	82.4	1.648	86.6	1.732
Flint	1.4	0.028	29.8	0.596		
Five Days						
Amber	15.4	0.308	81.3	1.626	86.6	1.732
Flint	0.6	0.012	16.2	0.324		
Seven Days						
Amber	5.0	0.100	79.6	1.592	86.6	1.732
Flint	0.0	0.000	8.1	0.162		
Ten Days						
Amber	3.8	0.076	77.8	1.556	86.2	1.724
Flint	...	.....	4.8	0.096		
Fifteen Days						
Amber	2.1	0.042	76.8	1.536	86.0	1.720
Flint	...	.....	3.5	0.070		
Twenty Days						
Amber	1.6	0.032	74.5	1.490	85.9	1.718
Flint	...	.....	2.2	0.024		
Thirty Days						
Amber	0.8	0.016	71.8	1.436	85.7	1.714
Flint	...	.....	0.5	0.010		
Sixty Days						
Amber	0.0	0.000	54.4	1.088	84.5	1.690

TABLE 67

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN 25 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 88.8 or 1.776 mcg./ml.						
One Day						
Amber	65.5	1.310	88.0	1.760	88.8	1.776
Flint	3.8	0.076	80.1	1.602		
Three Days						
Amber	35.5	0.710	87.1	1.742	88.8	1.776
Flint	3.1	0.062	61.2	1.224		
Five Days						
Amber	20.3	0.406	85.5	1.710	88.8	1.776
Flint	2.2	0.044	51.6	1.032		
Seven Days						
Amber	10.4	0.208	83.4	1.668	88.8	1.776
Flint	0.9	0.018	40.6	0.812		
Ten Days						
Amber	4.0	0.080	80.4	1.608	88.2	1.764
Flint	0.0	0.000	30.0	0.600		
Fifteen Days						
Amber	2.4	0.048	77.5	1.550	87.7	1.754
Flint	...	.....	14.7	0.294		
Twenty Days						
Amber	1.5	0.030	73.2	1.464	87.1	1.742
Flint	...	.....	7.5	0.150		
Thirty Days						
Amber	0.6	0.012	68.6	1.372	86.8	1.736
Flint	...	.....	2.7	0.054		
Sixty Days						
Amber	0.0	0.000	53.6	1.072	85.0	1.700

TABLE 68

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN 50 PER CENT GLYCERIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 90.0 or 1.800 mcg./ml.						
One Day						
Amber	67.0	1.340	89.1	1.782	90.0	1.800
Flint	4.0	0.080	81.0	1.620		
Three Days						
Amber	37.7	0.754	88.0	1.760	90.0	1.800
Flint	3.5	0.070	63.3	1.266		
Five Days						
Amber	25.2	0.504	85.4	1.708	90.0	1.800
Flint	2.6	0.052	52.2	1.044		
Seven Days						
Amber	16.7	0.334	83.8	1.676	90.0	1.800
Flint	1.0	0.020	41.1	0.822		
Ten Days						
Amber	9.5	0.190	82.4	1.648	89.7	1.794
Flint	0.2	0.004	29.9	0.598		
Fifteen Days						
Amber	7.0	0.140	81.6	1.632	89.1	1.782
Flint	0.0	0.000	15.2	0.304		
Twenty Days						
Amber	4.4	0.088	75.4	1.508	88.8	1.776
Flint	...	.....	7.8	0.156		
Thirty Days						
Amber	2.1	0.042	69.5	1.390	88.3	1.766
Flint	...	.....	2.9	0.058		
Sixty Days						
Amber	0.0	0.000	65.7	1.314	86.8	1.736

TABLE 69

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN 25 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 90.4 or 1.808 mcg./ml.						
One Day						
Amber	57.9	1.158	89.5	1.790	90.4	1.808
Flint	1.7	0.034	70.3	1.406		
Three Days						
Amber	24.5	0.490	88.3	1.766	90.4	1.808
Flint	1.5	0.030	55.8	1.116		
Five Days						
Amber	16.4	0.328	86.8	1.736	90.4	1.808
Flint	0.7	0.014	34.4	0.688		
Seven Days						
Amber	11.0	0.220	84.0	1.680	90.4	1.808
Flint	0.2	0.004	24.5	0.490		
Ten Days						
Amber	4.9	0.098	79.8	1.596	90.1	1.802
Flint	0.0	0.000	12.0	0.240		
Fifteen Days						
Amber	2.5	0.050	78.0	1.560	89.7	1.794
Flint	...	.....	7.0	0.140		
Twenty Days						
Amber	1.0	0.020	71.7	1.434	89.0	1.780
Flint	...	.....	4.8	0.096		
Thirty Days						
Amber	0.8	0.016	68.4	1.368	88.4	1.768
Flint	...	.....	2.1	0.022		
Sixty Days						
Amber	0.0	0.000	60.2	1.204	86.8	1.736

TABLE 70

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN 50 PER CENT PROPYLENE GLYCOL IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 89.5 or 1.790 mcg./ml.						
One Day						
Amber	63.8	1.276	88.6	1.772	89.5	1.790
Flint	2.0	0.040	74.4	1.488		
Three Days						
Amber	27.0	0.540	87.5	1.750	89.5	1.790
Flint	1.6	0.032	58.3	1.166		
Five Days						
Amber	14.8	0.296	86.4	1.728	89.5	1.790
Flint	0.7	0.014	37.2	0.744		
Seven Days						
Amber	9.8	0.196	85.1	1.702	89.5	1.790
Flint	0.3	0.006	26.3	0.526		
Ten Days						
Amber	4.5	0.090	83.2	1.664	89.5	1.790
Flint	0.0	0.000	15.4	0.308		
Fifteen Days						
Amber	2.6	0.052	82.3	1.646	89.0	1.780
Flint	...	.....	9.2	0.184		
Twenty Days						
Amber	1.6	0.032	78.3	1.566	88.7	1.774
Flint	...	.....	4.5	0.090		
Thirty Days						
Amber	1.0	0.020	69.5	1.390	88.2	1.764
Flint	...	.....	2.4	0.048		
Sixty Days						
Amber	0.0	0.000	61.4	1.228	86.7	1.734

TABLE 71

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN A SATURATED SOLUTION OF ETHYL AMINOBENZOATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 87.8 or 1.756 mcg./ml.						
One Day						
Amber	55.1	1.102	87.0	1.740	87.8	1.756
Flint	1.9	0.038	80.0	1.600		
Three Days						
Amber	32.0	0.640	86.1	1.722	87.8	1.756
Flint	1.4	0.028	63.5	1.270		
Five Days						
Amber	24.4	0.488	85.4	1.708	87.8	1.756
Flint	0.8	0.016	55.4	1.108		
Seven Days						
Amber	17.0	0.340	84.6	1.692	87.8	1.756
Flint	0.1	0.002	42.4	0.848		
Ten Days						
Amber	6.8	0.136	83.2	1.664	87.2	1.744
Flint	0.0	0.000	31.3	0.626		
Fifteen Days						
Amber	4.5	0.090	82.4	1.648	87.2	1.744
Flint	...	.....	20.4	0.408		
Twenty Days						
Amber	2.5	0.050	81.2	1.624	86.9	1.738
Flint	...	.....	13.8	0.276		
Thirty Days						
Amber	1.0	0.020	79.3	1.586	86.5	1.730
Flint	...	.....	5.5	0.110		
Sixty Days						
Amber	0.0	0.000	74.7	1.494	85.9	1.718

TABLE 72

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN 0.01 PER CENT QUININE BISULFATE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 89.2 or 1.784 mcg./ml.						
One Day						
Amber	62.3	1.246	88.8	1.776	89.2	1.784
Flint	2.1	0.042	81.0	1.620		
Three Days						
Amber	34.8	0.696	87.1	1.742	89.2	1.784
Flint	1.7	0.034	63.3	1.266		
Five Days						
Amber	26.8	0.536	86.0	1.720	89.2	1.784
Flint	0.9	0.018	44.3	0.886		
Seven Days						
Amber	15.2	0.304	84.2	1.684	89.2	1.784
Flint	0.2	0.004	39.8	0.796		
Ten Days						
Amber	3.9	0.078	82.7	1.654	89.2	1.784
Flint	0.0	0.000	26.1	0.522		
Fifteen Days						
Amber	2.8	0.056	80.1	1.602	89.2	1.784
Flint	...	.....	17.5	0.350		
Twenty Days						
Amber	1.7	0.034	77.8	1.556	88.8	1.776
Flint	...	.....	6.5	0.130		
Thirty Days						
Amber	0.9	0.018	73.7	1.474	88.3	1.766
Flint	...	.....	3.5	0.070		
Sixty Days						
Amber	0.0	0.000	67.1	1.342	87.1	1.742

TABLE 73

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN A SATURATED SOLUTION OF BETA-METHYL UMBELLIFERONE IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 88.5 or 1.770 mcg./ml.						
One Day						
Amber	61.0	1.220	87.2	1.748	88.5	1.770
Flint	2.1	0.042	70.2	1.404		
Three Days						
Amber	38.5	0.770	85.7	1.714	88.5	1.770
Flint	1.6	0.032	60.3	1.206		
Five Days						
Amber	20.3	0.406	84.3	1.686	88.5	1.770
Flint	0.6	0.012	49.9	0.998		
Seven Days						
Amber	6.3	0.126	82.2	1.644	88.5	1.770
Flint	0.0	0.000	43.7	0.874		
Ten Days						
Amber	4.2	0.084	80.6	1.612	88.5	1.770
Flint	...	.....	30.1	0.602		
Fifteen Days						
Amber	2.1	0.042	79.3	1.586	88.1	1.762
Flint	...	.....	20.5	0.410		
Twenty Days						
Amber	1.4	0.028	78.1	1.562	87.8	1.756
Flint	...	.....	7.2	0.144		
Thirty Days						
Amber	0.8	0.016	77.1	1.542	87.5	1.750
Flint	...	.....	4.4	0.088		
Sixty Days						
Amber	0.0	0.000	64.0	1.280	86.9	1.738

TABLE 74

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN 1.0 PER CENT UREA IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 86.9 or 1.738 mcg./ml.						
One Day						
Amber	67.1	1.342	85.3	1.706	86.9	1.738
Flint	2.0	0.040	58.1	1.162		
Three Days						
Amber	28.7	0.574	83.5	1.670	86.9	1.738
Flint	1.3	0.026	17.5	0.350		
Five Days						
Amber	10.0	0.200	78.0	1.560	86.9	1.738
Flint	0.8	0.016	13.0	0.260		
Seven Days						
Amber	7.1	0.142	76.1	1.522	86.9	1.738
Flint	0.0	0.000	8.8	0.176		
Ten Days						
Amber	5.0	0.100	61.0	1.220	86.5	1.730
Flint	...	.....	3.9	0.078		
Fifteen Days						
Amber	3.3	0.066	42.3	0.846	86.0	1.720
Flint	...	.....	1.6	0.032		
Twenty Days						
Amber	2.6	0.052	40.7	0.814	85.3	1.706
Flint	...	.....	0.4	0.008		
Thirty Days						
Amber	1.0	0.020	34.2	0.684	84.2	1.684
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	23.3	0.466	82.1	1.642

TABLE 75

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN 0.1 PER CENT TWEEN 80 IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 88.1 or 1.762						
One Day						
Amber	66.3	1.326	85.9	1.698	88.1	1.762
Flint	1.7	0.034	60.9	1.218		
Three Days						
Amber	36.1	0.722	84.5	1.690	88.1	1.762
Flint	1.0	0.020	18.0	0.360		
Five Days						
Amber	20.9	0.418	82.7	1.654	88.1	1.762
Flint	0.6	0.012	14.2	0.284		
Seven Days						
Amber	10.3	0.206	81.5	1.630	88.1	1.762
Flint	0.0	0.000	8.5	0.170		
Ten Days						
Amber	4.0	0.080	78.0	1.560	87.8	1.756
Flint	...	.....	3.7	0.074		
Fifteen Days						
Amber	2.4	0.048	76.3	1.526	87.5	1.750
Flint	...	.....	1.5	0.030		
Twenty Days						
Amber	1.7	0.034	71.2	1.424	87.2	1.744
Flint	...	.....	0.5	0.010		
Thirty Days						
Amber	1.1	0.022	67.9	1.358	86.5	1.730
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	48.8	0.976	85.0	1.700

TABLE 76

THE STABILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN OF 0.5 PER CENT NIACIN IN DISTILLED WATER STORED UNDER VARIOUS CONDITIONS IN FLINT AND AMBER BOTTLES

	Sunlight		Diffused Light		Darkness	
	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.	Lumetron Reading	Mcg./Ml.
Lumetron Reading of Freshly Prepared Sample: 84.5 or 1.690 mcg./ml.						
One Day						
Amber	52.2	1.044	82.6	1.652	84.5	1.690
Flint	2.5	0.050	52.4	1.048		
Three Days						
Amber	26.4	0.528	81.5	1.630	84.5	1.690
Flint	1.3	0.026	31.1	0.622		
Five Days						
Amber	13.3	0.266	79.2	1.584	84.5	1.690
Flint	0.5	0.010	20.3	0.406		
Seven Days						
Amber	3.5	0.070	77.4	1.548	84.5	1.690
Flint	0.0	0.000	5.0	0.100		
Ten Days						
Amber	1.8	0.036	76.6	1.532	84.2	1.684
Flint	...	.....	3.2	0.064		
Fifteen Days						
Amber	1.5	0.030	75.3	1.506	84.0	1.680
Flint	...	.....	2.0	0.040		
Twenty Days						
Amber	1.0	0.020	71.4	1.428	83.6	1.672
Flint	...	.....	1.0	0.020		
Thirty Days						
Amber	0.6	0.012	66.3	1.326	82.7	1.654
Flint	...	.....	0.0	0.000		
Sixty Days						
Amber	0.0	0.000	51.3	1.026	80.5	1.610

TABLE 77

THE SOLUBILITY OF A LEVULINIC ACID DERIVATIVE OF RIBOFLAVIN IN SOME AQUEOUS SOLUTIONS AND OTHER SOLVENTS

	Average Lumetron Reading	Mg./Ml.
Distilled Water	75.0	1.87
0.9% Sodium Chloride	77.5	1.93
0.9% Potassium Chloride	77.5	1.93
1.0% Sodium Acid Phosphate	75.0	1.87
1.0% Potassium Acid Phosphate	75.0	1.87
1.0% Niacinamide	87.2	2.17
1.0% Urea	80.0	2.00
Propylene Glycol	96.3	2.39
Glycerin	63.0	1.57
Alcohol	15.5	0.39

Results with a Citraconic Anhydride Derivative of Riboflavin

The citraconic anhydride derivative of riboflavin was prepared according to the procedure mentioned under preparation of derivatives.

This derivative was dark orange in color and crystalline. It was very hygroscopic. The melting point range was between 224-228° C. When dry, it was found not to be appreciably affected by diffused light.

Assay of the citraconic anhydride derivative of riboflavin showed a 76.5 per cent riboflavin content. Accordingly, 1 Gm. of riboflavin was equivalent to 0.765 Gm. of the derivative. This was determined fluorophotometrically.

Solubility studies were evaluated for the citraconic anhydride derivative of riboflavin in the same manner as that determined for the other derivatives.

TABLE 78

THE SOLUBILITY OF A CITRACONIC ANHYDRIDE DERIVATIVE OF RIBOFLAVIN IN SOME AQUEOUS SOLUTIONS AND OTHER SOLVENTS

	Average Lumetron Reading	Mg./Ml.
Distilled Water	76.0	1.99
0.9% Sodium Chloride	78.0	2.04
0.9% Potassium Chloride	78.0	2.04
1.0% Sodium Acid Phosphate	80.5	2.11
1.0% Potassium Acid Phosphate	80.5	2.11
1.0% Niacinamide	100.0	2.62
1.0% Urea	79.2	2.07
Propylene Glycol	63.5	1.66
Glycerin	52.0	1.36
Alcohol	25.2	0.66

DISCUSSION OF RESULTS

Although a number of derivatives of riboflavin were made in this investigation, only the pyruvic acid, levulinic acid and citraconic anhydride derivatives showed any promise with regard to an increase in solubility. This synthesis probably gave the following reaction in which an ester was formed:

$$ROH + R'COOH \longrightarrow R'COOR + H_2O.$$

ROH is represented by riboflavin as the 5' terminal alcohol group attached to the ribose portion of the molecule. R'COOH is represented by any organic acid used in the process of esterification.

In general, the results showed that there was a more rapid deterioration of the vitamin in those solutions stored in direct sunlight than in those placed in diffused light. In both sunlight and diffused light the stability was greater in an amber container rather than in one of flint.

It was interesting to note that in complete darkness all the riboflavin preparations investigated showed only a negligible loss in vitamin content over a sixty-day storage period. This would indicate that certain light waves were responsible for the destruction of the fluorescent portion of the molecule. In view of this, it appears that there should be no problem in the breakdown of solutions of riboflavin or any derivative thereof as long as light is totally excluded.

All solutions of riboflavin derivatives stored in flint bottles and placed in direct sunlight precipitated eventually out of solution. In all instances there was a color change from brilliant orange or yellow-orange to that of dark brown or olive. Solutions of riboflavin derivatives stored in flint bottles and placed in diffused light did not precipitate out of solution. With regard to color changes in this instance, however, all solutions of the derivatives lost their brilliancy and some developed a green or brown tint. In no case did a precipitate occur in solutions stored in amber bottles in either diffused light or in direct sunlight. Solutions stored in the dark in either flint or amber bottles remained clear and retained their original color after the sixty-day storage test.

Stability studies with riboflavin and derivatives in distilled water and in aqueous buffered solutions, stored in amber bottles and in diffused light, showed a gradual deterioration over a sixty-day exposure period. Those solutions stored in flint bottles in diffused light showed over a 50 per cent destruction of the riboflavin potency at the end of three days of storage. It was also noted that stability was favored at lower pH values. This is in agreement with the investigations of Conner and Straub (57).

Riboflavin was observed to be somewhat more stable in distilled water and in buffered solutions than any of the derivatives studied. It was evident that as the riboflavin molecule was altered the stability was affected, especially in aqueous or buffered solutions.

In direct sunlight, all solutions in distilled water as well as

those buffered at various pH values and stored in flint bottles exhibited almost complete destruction at the end of an exposure period of one day. With riboflavin solutions in amber bottles placed in direct sunlight, a rapid deterioration was observed with more than 50 per cent destruction of the vitamin content at the end of five days. Riboflavin seemed to stand up longer to the direct rays of the sun in amber bottles, when dissolved in distilled water and in buffered solutions at an acid pH, than any of the other derivatives studied.

Popular pharmaceutical solvents such as glycerin and propylene glycol in distilled water were also chosen as solvents for stability study. The pyruvic acid derivative and the levulinic acid derivative as well as riboflavin-5'-phosphate sodium seemed to exhibit a greater stability with solutions in amber bottles containing 50 per cent glycerin and propylene glycol than in those with 25 per cent of the same solution. In fact, the stability was greater here than that of solutions in distilled water and at various buffered pH values. The fact that part of the water was replaced with an organic solvent which, accordingly, decreased the amount of the aqueous phase, could have affected the degree of hydrolysis of the riboflavin derivatives.

With solutions of the levulinic acid and pyruvic acid derivatives of riboflavin in flint bottles stored in diffused light, replacing part of the aqueous phase with glycerin or propylene glycol seemed to delay the destruction of riboflavin initially. However, with longer exposure to diffused light, there was almost complete destruction of the vitamin content in these solutions.

Solutions of the levulinic acid and pyruvic acid derivatives stored in flint bottles and in which part of the aqueous phase had been replaced with either glycerin or propylene glycol exhibited almost complete destruction when stored in direct sunlight. However, the same solutions stored in amber containers were observed to possess a somewhat greater initial stability and then a rapid deterioration. In view of this, it appears that there should be no advantage in the use of aqueous solutions of glycerin or propylene glycol as solvents for riboflavin or any of its derivatives when stored in flint bottles and in direct sunlight.

Stability tests with riboflavin in an aqueous solution of glycerin and propylene glycol in flint bottles showed only a slight advantage over the use of distilled water as the solvent.

Ethyl aminobenzoate, quinine bisulfate and beta-methyl umbelliferone possess sun screening properties. Aqueous solutions of these were prepared and studied as solvents for riboflavin and its derivatives. It was felt that such substances might screen out certain light rays and thereby add to the stability of riboflavin preparations in solution. The selection of a suitable sun screening agent presents considerable difficulty. Here, such an ideal substance should have a desirable solubility in water and yet remain physiologically inactive.

With solutions of riboflavin or derivatives thereof stored in direct sunlight and in flint and amber bottles, the use of these sun screening agents proved to be of no advantage. With solutions stored in amber bottles and in the presence of diffused light, the presence of

these agents influenced considerably the stability of all solutions of riboflavin and its derivatives. In all cases, a saturated solution of ethyl aminobenzoate seemed to contribute most to the stability of the fluorescent portion of the molecule. A saturated solution of ethyl aminobenzoate was more favorable to the stability of the pyruvic acid and levulinic acid derivatives than solutions of propylene glycol and glycerin in distilled water. Thus, the significance of light causing the destruction of riboflavin must again be brought out as perhaps the most important single factor contributing to the deterioration of the vitamin in solution.

Urea and nicotinic acid are frequently used as solubilizers for riboflavin. Aqueous solutions of these were prepared and studied as solvents for riboflavin and its derivatives. These solutions, when stored in diffused light exhibited a marked instability in the presence of urea. This was probably due to the alkaline degradation products of urea plus the effect of light on these solutions. Aqueous solutions of urea exposed to sunlight in both amber and flint bottles exhibited rapid deterioration. It was interesting to note, however, that only a negligible amount of deterioration was observed with solutions of urea stored in total darkness. Riboflavin and riboflavin derivatives in an aqueous solution of nicotinic acid showed stability similar to that of solutions buffered at an acid pH.

The stability of riboflavin and its derivatives in an aqueous solution of polyoxyethylene sorbitan monooleate or Tween 80 was similar to that exhibited by solutions of the vitamin in distilled water. It

was felt that Tween 80 might enhance stability of the solutions because it is frequently used in oral vitamin preparations.

The phenomenon of fluorescence is inherent to riboflavin solutions and, accordingly, additional investigations should be made with the hopes of finding a suitable solvent and stabilizing agent. The answer to the stability of riboflavin and any of its derivatives might very well rest with the elimination of all harmful light rays which contribute to its deterioration. This could be accomplished with the selection of a proper container for solutions and the use of a sun screening agent.

The solubility of riboflavin in various aqueous solutions showed that in the presence of sodium chloride and potassium chloride the solubility was actually increased somewhat whereas in the presence of sodium acid phosphate and potassium acid phosphate the observed solubility seemed to decrease slightly as compared to distilled water. Riboflavin showed the greatest solubility in aqueous solutions of niacinamide and urea as compared to the other aqueous solutions.

Of all the solvents evaluated in the solubility study, riboflavin was the most soluble in glycerin. It was soluble to the extent of 0.84 mg. per ml. This is a solubility six times greater than that in distilled water. However, this is not sufficient to be of practical value in pharmaceutical preparations. Propylene glycol was the next best solvent studied, whereas riboflavin was practically insoluble in alcohol. Riboflavin was soluble in water to the extent of 0.14 mg. per ml.

Due to the unusually high solubility of riboflavin-5'-phosphate sodium, the solubility had to be determined by additional dilution in order to fall in the range of the lumetron. Even when placing 1 ml. of the saturated solution in enough distilled water to make 1000 ml., the observed fluorescence was greater than 2 mcg. per ml. Accordingly, further dilution was necessary and this was accomplished by placing in a red volumetric flask 10 ml. of the liter dilution made with each of the saturated solutions of glycerin and propylene glycol and diluting to 250 ml. with distilled water. The saturated solution of distilled water and the other aqueous solutions used as solvents also had to be further diluted by placing 1 ml. of the original liter dilution in a red volumetric flask and diluting to 250 ml. with distilled water. The saturated solution in alcohol required no additional dilution.

Results showed that in distilled water riboflavin-5'-phosphate sodium had a solubility of 35.71 mg. per ml. It was found to be slightly more soluble in aqueous solutions of sodium chloride and potassium chloride and somewhat less soluble in aqueous solutions of sodium acid phosphate and potassium acid phosphate than in distilled water. Glycerin, propylene glycol and alcohol were not as satisfactory solvents for riboflavin-5'-phosphate sodium as distilled water or the various aqueous solutions studied. The unusually high solvent effect with 1.0 per cent niacinamide and urea in distilled water was probably due to the increased solubility of the unesterified portion of the salt. Hoffmann-La Roche's salt has one of the highest solubilities of any riboflavin derivative on the market but in dilute solution it appears to be more sensitive to

light than pure riboflavin.

Solubility studies with Flavaxin Soluble or riboflavin sodium-sodium tetraborate also had to be further diluted to fall in the range of the lumetron. Aqueous solutions of urea and niacinamide were diluted by placing 10 ml. of the saturated solution, which was previously diluted to one liter, in a 250-ml. red volumetric flask and then adding a sufficient quantity of distilled water. Solubilities in propylene glycol and glycerin were treated in the same manner.

Results showed that the solubility of Flavaxin Soluble in propylene glycol and glycerin were unusually high. However, the solubility in alcohol was very slight. It is possible, however, to attain higher concentrations of riboflavin sodium-sodium tetraborate by prolonged heating and at higher temperatures. In general, borates are only slowly soluble and possess a greater solubility at elevated temperatures.

The solubilities of the pyruvic acid, levulinic acid and citraconic anhydride derivatives of riboflavin were also studied. These derivatives were found to be more soluble than riboflavin in water. They were all about fifteen times more soluble than riboflavin. Propylene glycol was also found to be better than glycerin as a solvent. It was observed that urea and niacinamide increased the concentrations of these derivatives in aqueous solutions. Sodium chloride and potassium chloride seemed to have a similar effect.

SUMMARY AND CONCLUSIONS

1. A search of the literature revealed that numerous methods have been suggested for preparing solutions containing a relatively high concentration of riboflavin. Most of these suggested methods do not show any increase in stability greater than that of the pure vitamin. One of the purposes of this investigation was to prepare more soluble derivatives and to evaluate the stability and solubility of these in various types of solutions. Solubility and stability studies were also evaluated for riboflavin, riboflavin-5'-phosphate sodium and Flavaxin Soluble.

2. The levulinic acid, pyruvic acid and citraconic anhydride derivatives of riboflavin were prepared. Solutions of these derivatives were placed in flint and amber bottles and stored in sunlight, diffused light and darkness. Solutions of riboflavin, riboflavin-5'-phosphate sodium and Flavaxin Soluble were also evaluated for stability in the same manner.

3. Amber bottles were much better than flint for maintaining the vitamin potency of solutions of riboflavin or any of its derivatives. Complete darkness caused only a negligible destruction of the vitamin with all types of solutions. A rapid deterioration occured with all solutions placed in direct sunlight irrespective of the type of container used.

4. Riboflavin and its derivatives were more stable at lower pH values. By replacing part of the aqueous phase with glycerin or propylene glycol, the stability of solutions of the derivatives prepared in this investigation and that of riboflavin-5'-phosphate sodium was better than solutions with distilled water. The use of some sun screening agents, especially a saturated aqueous solution of ethyl aminobenzoate, delayed destruction of the vitamin.

5. Solubility studies with riboflavin and some of its derivatives showed that riboflavin-5'-phosphate sodium had the highest solubility in water. The pyruvic acid, levulinic acid and citraconic anhydride derivatives showed a higher solubility in water than riboflavin. Flavaxin Soluble was slowly soluble in water and higher concentrations are possible at elevated temperatures.

6. The phenomenon of fluorescence is inherent to riboflavin solutions and, accordingly, additional investigations should be made with the hopes of finding a suitable solvent and/or stabilizing agent which would increase the stability of solutions to light.

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BIOGRAPHICAL ITEMS

Joel John Hertz was born in Palo Alto, California, on November 22, 1926. After graduation from high school, he attended Stanford University for two years before enlisting in the United States Army. While in Service, he was with the Medical Department for two years in Occupied Japan.

He entered the University of Florida in September, 1948, and received the degree of Bachelor of Science in Pharmacy, "with high honors," in February, 1951. In April, 1951, he was awarded the Lehn and Fink Gold Medal Award for excellence in pharmacy, pharmacognosy, and pharmacology.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Pharmacy and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 7, 1954

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